

Bacillus and Other Aerobic Endospore-Forming Bacteria*

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24

TAXONOMY

Bacillus was defined in 1920 as a genus of gram-positive, aerobic sporeformers, but since 1995 three strictly anaerobic and seven asporogenous *Bacillus* species have been proposed. This undermining of the definition has occurred because 16S rRNA gene sequence analysis has permitted the recognition of genus boundaries, whereas genera were previously defined phenotypically, as pragmatic collections of species sharing key (i.e., diagnostic) features. Many new species have been delineated largely on the basis of 16S rRNA gene sequence similarity and DNA-DNA relatedness—phenotypic descriptions may be brief, and routine phenotypic characters for distinguishing some species are very few and of little practical value.

With the accumulation of 16S rRNA gene sequence data, *Bacillus* has been divided; 14 new genera containing species originally allocated to *Bacillus* have been published since 1990, and together with *Bacillus* itself the species in these genera now number 388. In addition, 38 genera containing aerobic endospore formers not originally allocated to *Bacillus* have been described. *Sporosarcina* was proposed in 1936, but the other 37 genera have been proposed since 1990, and together they contain 109 species. Following some mergers, there are now 53 genera of aerobic endospore formers overall, and over 520 species. Although seven named families containing aerobic endospore formers now lie within the order *Bacillales* of the class “*Bacilli*,” in the phylum *Firmicutes*, only the families *Bacillaceae* and *Paenibacillaceae* contain species that may be of clinical interest.

The clinical bacteriologist need not, therefore, be greatly concerned at this taxonomic expansion, because for the medically relevant species it does not represent taxonomic upheaval. *Bacillus* is still the largest genus, with 162 species (90), and it continues to accommodate most of the best-known names such as *Bacillus subtilis* (the type species), *B. anthracis*, *B. cereus*, *B. licheniformis*, *B. megaterium*, *B. pumilus*, and *B. thuringiensis*. Relatively few other familiar names, some of which are of potential clinical interest, have been transferred to newer genera: *Brevibacillus brevis* (91), *Geobacillus stearothermophilus* (92; important as a thermophilic contaminant and autoclave

test organism), *Lysinibacillus sphaericus* (2), and *Paenibacillus polymyxa* and *Paenibacillus macerans*. However, several new *Bacillus* and *Paenibacillus* species have been proposed on the basis of single isolates of unknown significance from clinical sources: *B. idriensis* and *B. infantis* from neonatal sepsis (82); *P. konsidensis*, *P. massiliensis*, *P. sanguinis*, and *P. timonensis* from blood cultures (83, 116); *B. massiliensis* and *P. provencensis* isolated from cerebrospinal fluid (CSF) (56, 117); *P. turicensis* from a CSF shunt (14); and *P. urinalis* from urine (117). “*Paenibacillus hongkongensis*,” now classified in a new genus of aerobic endospore formers as *Cohnella hongkongensis* (74), was isolated from a case of pseudobacteremia in a boy with neutropenic fever.

DESCRIPTION OF THE GENERA

Notwithstanding the handful of strictly anaerobic and asporogenous members of *Bacillus*, those species likely to be isolated in a clinical laboratory are rod-shaped, endospore-forming organisms that may be aerobic or facultatively anaerobic, and young cultures are usually gram positive but sometimes gram variable or clearly gram negative. They are mostly catalase positive and may be motile by means of peritrichous flagella. Most species are mesophilic, but there are some thermophilic and psychrophilic species. A large number of the other, newer genera comprise small numbers of species that are unlikely to be encountered in a clinical laboratory, as many of them are from exotic and extreme environments.

EPIDEMIOLOGY AND TRANSMISSION

Most aerobic endospore formers are saprophytic organisms living in the natural environment, and many species are very widely distributed. Some species, however, are opportunistic or obligate pathogens of animals, including humans, other mammals, and insects. The main habitats are soils of all kinds, ranging from acid to alkaline, nonsaline to highly saline, hot to cold, and fertile to desert, and the water columns and bottom deposits of fresh and marine waters. They have been isolated from air at high altitude, from subterranean waters, and from volcanic and permafrost soils. Endospores readily survive distribution in soils, dusts, and aerosols from natural environments to a wide variety of other habitats such as man-made environments, and they may be carried across the globe by wind. The resistance of the spores to

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heat, radiation, disinfectants, and desiccation also results in aerobic endospore formers being frequent contaminants in the operating room, on surgical dressings, in pharmaceutical products, and in foods. Dried foods such as spices, milk powders, and farinaceous products are often quite heavily contaminated with spores, and when water becomes available during food preparation these spores may germinate, leading to spoilage or food poisoning. *B. anthracis* is, to all intents and purposes, an obligate pathogen of animals and humans. Its close relative *B. cereus* is well established as an opportunistic pathogen, and other aerobic endospore formers can also be opportunistic pathogens occasionally. Three members of the *B. cereus* group—*B. anthracis*, *B. cereus*, and *B. thuringiensis*—are regarded by many as pathovars of a single species, and there is evidence of extensive horizontal gene transfer within the *B. cereus* group (18).

Anthrax was the first example of an infection of humans and animals proven as caused by a bacterium, and this disease remains the most widely recognized clinical condition caused by a *Bacillus* species. It is primarily a disease of domestic or wild animals, and prior to the availability of an effective veterinary vaccine in the late 1930s, anthrax was one of the foremost causes worldwide of mortality in cattle, sheep, goats, and horses. Humans almost invariably contract anthrax directly or indirectly from animals. Over the past half century there has been a marked decline in the incidence of anthrax in both animals and humans; the use of veterinary and human vaccines, improvements in factory hygiene, sterilization procedures for imported animal products, and the increased use of man-made alternatives to animal hides or hair have all contributed to this. Nevertheless, the disease continues to be endemic in many countries, particularly those that lack efficient vaccination policies. It is still common in several sub-Saharan African countries, western China, some Mediterranean countries, small pockets in Canada and the United States, and certain countries of central Asia and central and South America. National vaccination programs have led to a progressive global reduction in livestock cases over the last 30 years, but there are concomitant problems of diminishing veterinary experience and a public ignorance of the disease; the former problem may lead to individual outbreaks taking longer to recognize and control, and the latter to the sale and slaughter of affected animals (140). Direct animal-to-animal transmission (i.e., excluding scavengers feeding on carcasses of animals with anthrax) is very rare. Because *B. anthracis* spores remain viable in soil for many years and their persistence does not depend on animal reservoirs, *B. anthracis* is exceedingly difficult to eradicate from an area of endemicity, and regions of nonendemicity must be constantly on the alert for the arrival of *B. anthracis* in imported animal products. Anthrax is not contagious, and transmission to humans is usually restricted to direct contact with infected animals, or products from infected animals; however, there have been reports of person-to-person transmission of cutaneous anthrax, including a case of a mother with a cutaneous lesion on her finger infecting her 1-year-old child, and a case of transmission of cutaneous abdominal lesions from one brother to another brother who shared a bed (146). Contaminated fomites, including such oddities as a communal loofah, or infection of an injection site by contamination of the skin or by syringe have also rarely been implicated (86), as has person-to-person, nosocomial spread from an umbilical infection (151).

The continued existence of *B. anthracis* in the ecosystem appears to depend on a periodic multiplication phase within an animal host, and so it is generally regarded as an obligate

pathogen. Its environmental presence thus reflects contamination from an animal source at some time and persistence of spores, rather than replication within the soil. However, some authorities believe that self-maintenance may occur within certain soils, and germination of *B. anthracis* spores and genetic exchange between vegetative cells growing in grass rhizosphere have been demonstrated (119). In human and animal specimens the organism is usually sought only when the case history suggests that it is reasonable to suspect anthrax.

B. anthracis has been subjected to military research, development, and occasional deployment in several different countries over many years, following attacks on livestock during World War I, and it has remained high on the list of agents that could be used in biological warfare or bioterrorism. Few reports of laboratory-acquired infections exist, but a major outbreak occurred in April 1979 in the city of Sverdlovsk, USSR (now Yekaterinburg, Russia), in the Urals as a result of the accidental release of spores from a military production facility. Although the natural disease is readily controllable, the 2001 bioterrorism-related anthrax outbreak in the United States increased the public concern about this disease (see chapter 12).

CLINICAL SIGNIFICANCE

The majority of aerobic endospore-forming species apparently have little or no pathogenic potential and are rarely associated with disease. The principal exceptions to this are *B. anthracis*, the agent of anthrax, and *B. cereus*, but a number of other species, particularly *B. licheniformis* and *B. pumilus*, have been implicated in food poisoning and other human and animal infections.

Bacillus anthracis

Human anthrax has traditionally been classified as either (i) nonindustrial, resulting from close contact with infected animals or their carcasses after death from the disease, or (ii) industrial, as acquired by those employed in processing wool, hair, hides, bones, or other animal products. Dependent on the route of infection, there are three major clinical forms of anthrax: cutaneous, inhalation, and ingestion or oral-route anthrax. Anthrax meningitis can develop as a complication of any of these forms, or occasionally in the absence of any of them (57, 121).

Cutaneous anthrax accounts for about 99% of naturally acquired human anthrax cases worldwide; an estimated 2,000 cases are reported annually. Infection typically occurs when spores are inoculated through a break in the skin, although case reports and animal studies suggest that preexisting lesions are not necessary for infection (60); infections following insect bites have been reported. Following an incubation period of usually 2 to 6 days (but with extremes of a few hours to 3 weeks) a small papule appears, progressing over the next 24 h to a ring of vesicles, with subsequent ulceration and formation of a characteristic blackened eschar. Subsequent eschar formation may become thick and surrounded by extensive edema. Fever, pus, and pain at the site are normally absent; their presence probably indicates secondary bacterial infection. Before the availability of antimicrobial therapy, 10 to 20% of untreated cases of cutaneous anthrax were fatal. Less than 1% of cases are fatal today, and they are mainly due to obstruction of the airways by the edema that accompanies lesions on the face or neck or as a result of progression of the cutaneous disease into systemic infection. Eschars take several days to evolve, and even with effective antimicrobial therapy they may take several weeks to resolve.

Ingestion anthrax is not uncommon in regions of endemicity worldwide where socioeconomic conditions are poor and people eat raw or undercooked meat of anthrax-infected animals that have died suddenly; asymptomatic infections and symptomatic infections with recovery may not be uncommon (124). It takes two forms: oral or oropharyngeal infection, in which the lesion is in the buccal cavity or on the tongue, tonsils, or posterior pharyngeal wall, and gastrointestinal anthrax, in which ulcerations develop anywhere within the tract, but primarily in the mucosae of the terminal ileum or cecum. Oropharyngeal symptoms include sore throat, dysphagia, and regional lymphadenopathy, followed by severe edema of neck and chest. Intestinal anthrax causes nausea, vomiting, anorexia, abdominal pain, mild diarrhea, and fever; this can progress to bloody diarrhea, hematemesis, and massive ascites (140). Mortality rates range widely, owing to the nonspecific nature of the early symptoms and late initiation of antimicrobial therapy.

Between 1900 and 2001, only 18 cases of naturally acquired inhalation anthrax were recorded in the United States, with 16 (89%) being fatal (16), and figures in the United Kingdom show a similar picture. Recently, however, anthrax has occurred in persons using contaminated imported animal hides for drum making. A case of inhalation anthrax in an African drum maker in 2006 was the first naturally occurring case of inhalation anthrax in the United States since 1976; the hides from west Africa were contaminated with *B. anthracis* spores. Two cases of cutaneous anthrax, in a drum maker and a family member, occurred in the United States in 2007 (23), and there was a fatal case of inhalation anthrax in a drum maker in the United Kingdom in 2008 (3). Both cutaneous and inhalation anthrax have also been associated with the handling or playing of goatskin drums contaminated with spores of *B. anthracis* (40). Among the 22 cases of the anthrax outbreak in the United States in late 2001, in which spores were delivered in mailed letters, early recognition and treatment of the 11 patients with confirmed inhalation anthrax resulted in a 65% survival rate (9).

The inhaled spores are ingested by macrophages, but the role of macrophages thereafter is unclear, as they may be bactericidal towards germinated spores but also be crippled by lethal and/or edema toxin and so allow bacterial outgrowth (31). The replacement of the older name for this form of the disease, "pulmonary anthrax," with the newer name "inhalation anthrax" is a reflection of the fact that active infection occurs in the lymph nodes rather than the lungs themselves. Analysis of 10 of the cases associated with the bioterrorism events of 2001 (71) revealed a median incubation period of 4 days (range, 4 to 6 days). All of the patients with inhalation anthrax had severe illness and were hospitalized. Their clinical presentation included fever or chills, fatigue or malaise, minimal or nonproductive cough, dyspnea, and nausea or vomiting; some had chest pain and sweats. All patients had abnormal chest radiographic images, with pleural effusion, infiltrates, or mediastinal widening.

Regardless of the form of the disease, the generalized symptoms that may initially be mild (fatigue, malaise, fever, and/or gastrointestinal symptoms) can rapidly develop into a fulminant state following lymphohematogenous spread from a primary lesion, with symptoms of dyspnea, cyanosis, severe pyrexia; and disorientation, followed by circulatory failure, shock, coma, and death (71). Depending on the host, there is a rapid buildup of the bacteria in the blood over the last few hours to terminal levels of 10^7 to 10^9 /ml in the most susceptible species. Enhanced clinical and laboratory expertise and conducting prospective surveillance

are critical components of rapid anthrax diagnosis and for response preparedness, should *B. anthracis* be used in bioterrorist attacks (52).

***Bacillus cereus* Group**

Bacillus cereus is a ubiquitous organism, and clinical isolates are phylogenetically diverse (68); it is a wide-ranging opportunistic pathogen of humans and other animals and an important cause of foodborne illness. *B. cereus* caused 21 (2%) mean annual total foodborne outbreaks and 160 (1%) mean annual total foodborne illnesses between 2001 and 2005 in the United States as reported through the Foodborne Diseases Active Surveillance Network (FoodNet) of the Centers for Disease Control and Prevention's (CDC's) Emerging Infections Program. However, this probably underrepresents the true burden of illness, since outbreaks involving less commonly identified pathogens, such as *B. cereus*, are less likely to have a confirmed etiology because these organisms are not always considered in clinical, epidemiological, and laboratory investigations of foodborne disease outbreaks (24).

B. cereus is the etiological agent of two distinct food poisoning syndromes. The diarrheal type is characterized by abdominal pain and diarrhea 8 to 16 h after ingestion of contaminated food; it is associated with a wide diversity of foods, from meats and vegetable dishes to pastas, desserts, cakes, sauces, and milk. The emetic type is characterized by nausea and vomiting 1 to 5 h after eating the offending food; oriental rice dishes are predominant sources, although occasionally other foods such as pasteurized cream, milk pudding, pastas, and reconstituted formulas have been implicated. One emetic outbreak followed the mere handling of contaminated rice in a children's craft activity, and fulminant liver failure associated with the emetic toxin has been reported (94). *B. cereus* spores are very adherent to surfaces and are widespread in food preparation environments, and they can survive many cleaning and normal cooking procedures. These are key factors for both syndromes; under conditions of improper food storage after cooking, the spores germinate and the vegetative cells multiply.

The toxigenic basis of *B. cereus* food poisonings and other *B. cereus* infections have been much elucidated over the last three decades. In diarrheal illness, one or more enterotoxins are produced by vegetative cells in the small intestine, following the ingestion of spores or vegetative cells; the toxins are believed to cause diarrhea by damaging the integrity of ileal epithelial cell membranes. *B. cereus* produces a range of protein toxins, of which three have been implicated in diarrheal illness: hemolysin BL (Hbl) and nonhemolytic enterotoxin (Nhe), both of which are three-component toxins restricted to the *B. cereus* group, and the single-component β -barrel, pore-forming cytotoxin K (CytK). These toxins may act synergistically, but there is evidence that Nhe is the most dominant in diarrheal illness (128). The emetic illness is an intoxication caused by a highly heat-, proteolysis-, and acid-resistant toxin preformed in the food—hence its rapid onset. The toxin, cereulide, is a small ring-formed dodecadeptide produced at the end of logarithmic growth, and its genetic determinants are borne on a plasmid related to the pXO1 virulence plasmid of *B. anthracis* (41). Cereulide production by *B. cereus* has not been demonstrated at temperatures below 12°C, but the related and psychrotolerant species *Bacillus weihenstephanensis* may produce detectable cereulide at 8°C (135). Gherlardi et al. (53) used PCR amplification of toxin genes in conjunction with randomly amplified polymorphic DNA (RAPD)-PCR and multiplex RAPD-PCR to trace the source of two outbreaks of food poisoning.

Bacillus cereus is an important ocular pathogen, causing a rapidly progressive endophthalmitis that is refractory to treatment. Cases are, fortunately, infrequent; they usually occur after penetrating trauma of the eye but sometimes follow hematogenous spread or, occasionally, eye surgery. Significant loss of vision, and often loss of the eye itself, can occur within 24 to 48 h (101). *B. cereus* keratitis associated with contact lens wear has also been reported (109). Other *B. cereus* infections occur mainly, though not exclusively, in persons predisposed by neoplastic disease, immunosuppression, alcoholism and other drug abuse (including cases associated with contaminated heroin), the presence of catheters (65) or implants such as fluid shunts, or some other underlying condition such as diabetes, and fatalities occasionally result. Reported conditions include bacteremia, septicemia, fulminant sepsis with hemolysis, meningitis (following allogeneic stem cell transplants in two cases) (58), brain hemorrhage, ocular infections including panophthalmitis, ventricular shunt infections, endocarditis (1), pneumonia (47), pseudomembranous tracheobronchitis, exacerbation of bronchiectasis, empyema, pleurisy, peritonitis in a dialysis patient, lung abscess, brain abscess, liver abscess, osteomyelitis, salpingitis, urinary tract infection, and primary cutaneous infections. El Saleeby et al. (42) found an association between tea drinking and invasive *B. cereus* infection in immunocompromised children. Ko et al. (84) reported the emergence of a β -lactam-dependent strain associated with prolonged administration of β -lactams via an indwelling catheter in a neutropenic patient. Strains of *B. cereus* have been isolated in association with periodontitis (63). Wound infections, often of open fractures (38), and mostly in otherwise healthy persons, have been reported following surgery (associated, in one report, with contaminated incontinence pads), road traffic and other accidents, scalds, burns, plaster fixation, drug injection, and close-range gunshot and nail bomb injuries; some became necrotic and gangrenous (32). Fatal neonatal meningitis was caused by a blank firearm injury; blank cartridge propellants are commonly contaminated with the organism. Neonates also appear to be particularly susceptible to *B. cereus*, especially with umbilical stump infections, and cases of meningitis have been associated with the use of catheters and manual ventilation balloons (44). Respiratory tract infections have also been associated with contaminated ventilation systems among neonates and in a pediatric intensive care unit (73). Several cases of *B. cereus* pneumonia in metal workers and welders have been reported, including fatal infections (67). Infection of these metal workers may be related to welding-fume exposure, demonstrated to suppress pulmonary defenses in animal studies. Interestingly, the isolate from one of these cases was found to harbor a plasmid (pBCXO1) that was 99.6% similar to the pXO1 virulence plasmid of *B. anthracis*, while other isolates were also found to harbor either pXO1 virulence genes or both pXO1 and pXO2 virulence genes (67); the role of these pXO1 or pXO2 plasmid genes, if any, in the virulence of these isolates is not known. Additional atypical *B. cereus* strains, originally identified as *B. anthracis* due to the presence of pXO1 and pXO2 plasmids, have also been isolated in association with fatal infections of chimpanzees and a gorilla in Ivory Coast and Cameroon (79, 85). A nosocomial outbreak of *B. cereus* infection related to catheter use was associated with reused towels (35), and a hospital pseudo-outbreak was associated with contaminated ethanol used as a skin disinfectant. *B. cereus* also causes infections in domestic animals. It is a well-recognized agent of mastitis and abortion in cattle, particularly when animals are in winter housing, and can cause these conditions in other livestock.

Strains of *B. thuringiensis* commonly carry genes for *B. cereus* enterotoxins, and assays have demonstrated that enterotoxin production occurs, but the cereulide synthetase gene has not been detected in this species (6, 105). *B. thuringiensis* is well known as an insect pathogen, preparations of certain strains are widely used as biopesticides, and transgenic crop plants that express the insecticidal crystal toxin genes have been developed, but there is as yet no evidence of infections directly associated with the use of this organism as an insecticide. Occupational exposure to the organism has been connected with presence of the organism in feces but without gastrointestinal symptoms. A review of the safety of using *B. thuringiensis* as a biopesticide on crop plants found that the main pesticide strains assayed produced low titers of enterotoxin (13). There have been few reports of gastroenteritis outbreaks in which *B. thuringiensis* was implicated (98, 128). However, cases of such illness caused by *B. thuringiensis* may have been attributed to *B. cereus*, as the former may not produce its characteristic insecticidal toxin crystals when incubated at 37°C, owing to the loss of the plasmids carrying the relevant genes. There have been reports of wound, burn, and ocular infections with *B. thuringiensis*, and in a fatal case of pulmonary disease and bacteremia in a neutropenic patient, there was evidence that membrane-damaging toxins contributed to the infection (54). Experimental *B. thuringiensis* endophthalmitis has been achieved in rabbits (17).

Other Species

Reports of infections with non-*B. cereus* group species are comparatively rare but very diverse, and there have been several hospital pseudoepidemics associated with contaminated blood culture systems. *B. licheniformis* has been reported from prosthetic-valve endocarditis, pacemaker wire infection, ventriculitis following the removal of a meningioma, brain abscesses, septicemia following arteriography, bacteremia associated with indwelling central venous catheters, bacteremia during pregnancy with eclampsia and acute fibrinolysis, peritonitis in patients undergoing continuous ambulatory peritoneal dialysis (CAPD) and in a patient with volvulus and small-bowel perforation, ophthalmitis, and corneal ulcer after trauma. An outbreak of bacteremia among patients with blood malignancies was related to nonsterile cotton wool used during skin disinfection (106). *B. licheniformis* bacteremia has been reported for an immunocompetent man and has twice been reported in association with Munchausen's syndrome: one case followed self-inoculation with organic drain cleaner (61), and in another case following self-inoculation with soil, *B. pumilus* and *Paenibacillus polymyxa* were also isolated (51). *B. licheniformis* can cause foodborne diarrheal illness, and in a case associated with an infant fatality, lichenilysin A, a heat-stable cyclic lipopeptide, has been implicated (99). This organism is frequently associated with bovine abortion and has been shown to have a tropism for the bovine placenta; it has also been associated with abortion in water buffalo and occasionally with bovine mastitis. As with such *B. cereus* infections, these types of *B. licheniformis* infection are associated with wet and dirty conditions during winter housing, particularly when the animals lie in spilled silage, and in one outbreak a water tank contaminated with *B. licheniformis* was implicated.

The name *B. subtilis* was often used in the past for any clinical isolate, but since 1970 there have been reports of infection in which this species appears to have been identified accurately. They include cases of pneumonia, bacteremia, and septicemia associated with neoplastic disease;

breast prosthesis and ventriculo-atrial shunt infections; isolations from surgical wound drainage sites; endocarditis in a drug abuser; meningitis following a head injury; bacteremia associated with trauma; bacteremia in cancer patients; cholangitis associated with kidney and liver disease; and isolation from dermatolymphangioadenitis associated with filarial lymphedema. Two cases of severe hepatotoxicity followed the ingestion of nutritional supplements contaminated with *B. subtilis* (130). Administration of an oral probiotic preparation marketed for the treatment or prevention of intestinal disorders, and allegedly containing *B. subtilis*, led to a fatal septicemia in an immunocompromised patient; subsequently, the organism concerned was identified as *Bacillus clausii* (127). These authors reported another *B. clausii* infection, cholangitis in polycystic kidney disease, in a 15-year-old French boy who had undergone a renal transplant, but the source of the organism was unclear. *B. subtilis* has also been associated with cases of bovine mastitis and ovine abortion. *B. subtilis* has been implicated in foodborne illness: vomiting has been the commonest symptom, but with accompanying diarrhea frequently reported, the onset periods have been short (ranging from 10 min to 14 h; median, 2.5 h), the bacterial loads of the organism were high (10^5 to 10^9 CFU/g), and the implicated foods were often prepared dishes in which meat or fish was served with cereal-based components such as bread, pastry, rice, or stuffing. *Bacillus amyloliquefaciens*, a close relative of *B. subtilis*, is widely used industrially for enzyme and amino acid production, but human consumption of L-tryptophan manufactured in an organism genetically engineered from a strain of this species was associated with a large epidemic of eosinophilia-myalgia syndrome with 37 deaths; the causative agent has not been identified with certainty (100). Environmental strains of this species producing a heat-stable, nonprotein toxin have been isolated in association with building-related health problems (100).

Organisms identified as *B. circulans* have been isolated from cases of bacteremia in cancer patients, meningitis, CSF shunt infections, endocarditis, a wound infection in a cancer patient, a bite wound, endophthalmitis, peritonitis in a patient undergoing CAPD (12), and epidemic endophthalmitis associated with a contaminated product used during cataract surgery. It must be noted, however, that many isolates previously regarded as *B. circulans* might have been wrongly identified (see comments on *B. circulans* below). *B. coagulans* has been isolated from corneal infection, bacteremia, and bovine abortion. *B. pumilus* has been found in cases of cutaneous, pustule, and rectal fistula infections, central venous catheter infection in an immunocompetent child (10), bacteremias in immunosuppressed patients (106), bacteremia in a patient with Munchausen's syndrome (51), and repeated contamination of a blood platelet screening procedure; it has also been found in association with bovine mastitis. Toxigenic strains of *B. pumilus* have been isolated in association with foodborne illness and from clinical and environmental specimens, and a heat-stable cyclic lipopeptide, pumilacidin, was implicated in a rice-associated food poisoning outbreak (50). *Lysinibacillus sphaericus* has been implicated in a fatal lung pseudotumor, bacteremia, and meningitis. *B. megaterium* (eight isolates), *B. pumilus* (six), *Brevibacillus brevis* (five), *B. licheniformis* (two), and *B. subtilis* (one) isolated from chewing tobacco were found to produce potent exogenous virulence factors that caused plasma exudation and tissue dysfunction in an animal model (118). *B. megaterium* caused a delayed-onset lamellar keratitis following laser-assisted eye surgery (114).

Bacillus brevis has been isolated from corneal infection and implicated in several incidents of food poisoning; since those reports, the species was split (see "Taxonomy" above) and transferred to the new genus *Brevibacillus*. Strains of one of the newer species, *Brevibacillus agri*, were isolated in association with an outbreak of waterborne illness in Sweden; *Brevibacillus centrosporus* was isolated from a bronchoalveolar lavage fluid sample, *Brevibacillus parabrevis* was found in a breast abscess, and both species have been isolated from human blood (93). *Brevibacillus laterosporus* has been reported in association with a severe case of endophthalmitis.

Paenibacillus alvei has been isolated from cases of meningitis, endophthalmitis, a prosthetic hip infection in a patient with sickle cell anemia, wound infections, and, in association with *Clostridium perfringens*, a case of gas gangrene. *P. macerans* has been isolated from a wound infection following removal of a malignant melanoma, from a brain abscess following penetrating periorbital injury, from a catheter-associated infection in a leukemic patient, and from bovine abortion, and *P. polymyxa* has been isolated from bacteremia in a patient with cerebral infarction, a bacteremic case of Munchausen's syndrome (51), and ovine abortion. *Paenibacillus popilliae* has been reported from endocarditis, and *Paenibacillus larvae* has been reported from infection of a CSF shunt system.

Single isolations of several new *Bacillus* and *Paenibacillus* species from clinical specimens, but of unknown significance, are mentioned in "Taxonomy" above.

COLLECTION, TRANSPORT, AND STORAGE OF SPECIMENS

Bacillus species normally survive transport in freshly collected specimens or in a standard transport medium. Local transport of specimens (for no longer than a few hours) can be done at room temperature or at 2 to 8°C for most specimens, including serum. Generally, if specimens such as stool, sputum, pleural fluid, blood, and material on swabs are to be shipped overnight or longer, they should be sent at 2 to 8°C, while fresh tissue and serum samples should be shipped frozen. Formalin-fixed tissues can be sent at room temperature primarily for detection using immunohistochemistry and (although they are much less suitable) PCR. For blood specimens with which PCR will be used to detect *B. anthracis* DNA, collection tubes containing EDTA or citrate as an anticoagulant are preferable to those containing heparin.

All the clinically significant isolates reported to date are of species that grow, and often sporulate, on routine laboratory media at 37°C. It seems unlikely that many clinically important but more fastidious strains are being missed for the want of special media or growth conditions. Maintenance is simple if spores can be obtained, but it is a mistake to assume that a primary culture or subculture on blood agar will automatically yield spores if it is stored on the bench or in the incubator. It is best to grow the organism on nutrient agar (or Trypticase soy agar) containing 5 mg/liter manganese sulfate for a few days, and refrigerate when microscopy shows that most cells have sporulated. For most species, sporulated cultures on slants of this medium, sealed after incubation, can survive in a refrigerator for years. Alternatively, cultures (preferably sporulated) can be frozen or lyophilized.

Safety Aspects

Clinical specimens for isolation of *Bacillus* species other than *B. anthracis* can be handled safely on the open bench without special precautions. Efforts should be made to

avoid methods that produce aerosols. Any procedures that have the potential to generate aerosols should be done in a microbiological safety cabinet. Biosafety level 2 practices, containment equipment, and facilities are recommended for activities using clinical materials and diagnostic quantities of infectious cultures.

Isolation and presumptive identification of *B. anthracis* can be performed safely in the routine clinical microbiology laboratory, provided that normal good laboratory practice is observed. Preexposure vaccination is not recommended for laboratory personnel doing routine processing of clinical or environmental specimens in general diagnostic laboratories (26); however, biosafety level 3 facilities are recommended for all such work (140). When working with pure cultures of *B. anthracis*, direct and indirect contact of broken skin with cultures and contaminated laboratory surfaces, accidental parenteral inoculation, and, rarely, exposure to infectious aerosols are the primary hazards to laboratory personnel. Laboratories that frequently centrifuge *B. anthracis* suspensions should use an aerosol-tight rotor that can be repeatedly autoclaved (26).

Human infectious doses have not been established for *B. anthracis*; the U.S. Department of Defense estimates that a 50% lethal dose for humans is 8,000 to 10,000 spores, but this is largely based on data from nonhuman primate studies. When collecting clinical specimens for suspected anthrax, appropriate personal protective equipment should be used, such as disposable gloves, disposable apron or overalls, and boots which can be disinfected after use; for dusty samples that might contain many spores, the use of a face shield and/or a respirator should be considered. Full details of personal protective equipment and of disinfection and decontamination are given in Annexes 1 and 3 of the WHO *Anthrax in Humans and Animals* guidelines (140). It should be noted that although hand washing with soap and water or with chlorhexidine gluconate, and the use of hypochlorite-releasing towels, may reduce endospore contamination of the skin, waterless rubs containing ethanol are ineffective at removing endospores. Preexposure vaccination recommendations by Advisory Committee on Immunization Practices (ACIP) for laboratory exposures are addressed in "Vaccination" below.

Bacillus anthracis is defined as a select agent and is included on both the Department of Health and Human Services/CDC and U.S. Department of Agriculture/APHIS select agent lists. Thus, possession of the agent in the United States requires registration of the laboratory with either the CDC or APHIS. When *B. anthracis* is identified by a laboratory, the identification of this agent must be reported to the CDC or APHIS immediately and a APHIS/CDC Form 4 (Report of the Identification of a Select Agent or Toxin) submitted within 7 days. Other authorities should be notified as required by federal, state, or local laws. When *B. anthracis* is isolated in an unregistered laboratory, the organism must either be destroyed on-site by a recognized sterilization or inactivation process or be transferred to a registered laboratory within 7 days. Shipping of this agent requires completion of the APHIS/CDC Form 2 (Request to Transfer Select Agents and Toxins) and prior approval from either the CDC or APHIS (see http://www.cdc.gov/od/sap/final_rule.htm or <http://www.selectagents.gov/formsOverview.htm> for additional information of select agent regulation in the United States).

Specimens from Patients Suspected To Have Anthrax

In all cases, specimens from possible sources of infection (carcass, hides, hair, bones, etc.) should be sought in addition to patient specimens. Leakproof containers, to be placed in secondary containers for "double-bagging," and then secure,

outer containers for carriage are needed. A blood smear may reveal the capsulated rods or, if treatment has started, capsule "ghosts." Postmortem blood collected by venipuncture (a characteristic of anthrax is nonclotting blood at death) should be examined by smear (for capsule) and culture.

Guidelines for clinical evaluation of persons with possible anthrax can be found at <http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5043a1.htm>.

Cutaneous Anthrax

The edge of an eschar should be lifted and two specimens of vesicular fluid collected (preferably prior to initiation of antimicrobial therapy) by rotating swabs beneath it; one is for a smear for visualizing the capsule, Gram stain, and culture, and the other is for PCR. For immunohistochemical (IHC) analysis of cutaneous lesions, a full-thickness punch biopsy specimen fixed in 10% buffered formalin from a papule or vesicle lesion and including adjacent skin should be taken. Biopsies should also be taken from both vesicle and eschar if present (122).

Inhalation Anthrax

Anthrax will be suspected only if the patient's history suggests it. Chest radiographs and chest computed tomography scans are recommended. In addition to imaging studies, obtain blood cultures prior to antimicrobial therapy. Pleural fluid, if present, should be obtained for Gram stain, culture, and PCR. Serology is also useful for the diagnosis of cases when culture fails owing to previous treatment. Collect acute- and convalescent-phase serum samples 2 to 4 weeks apart for serologic testing. Pleural and/or bronchial biopsy samples can be tested by immunohistochemistry. Postmortem, the approach given for ingestion anthrax should be followed (see below).

Ingestion Anthrax

As with the inhalation form, anthrax is suspected only if an adequate history of the patient is known. Specimens from oral lesions may be collected in the same way as for the cutaneous disease (140). Premortem, obtain blood cultures before antimicrobial therapy. Ascites fluid, if present, should be collected for Gram stain, culture, and PCR. A stool or rectal swab, and material from any oropharyngeal lesions, should also be collected for Gram stain, culture, and PCR. Acute- and convalescent-phase serum samples, with the first obtained within 7 days of onset and the convalescent-phase sample obtained 2 to 3 weeks later, should be collected for serologic testing. Postmortem blood collected by venipuncture should be examined by smear (for observation of capsule) and culture. Any hemorrhagic fluid from the nose, mouth, or anus should be cultured. If these are positive, no further specimens are needed. Again, if negative, specimens of peritoneal or ascitic fluid, spleen, and/or mesenteric lymph nodes, aspirated by techniques avoiding spillage of fluids, may be collected for smear and culture.

Anthrax Meningitis

Collect CSF and blood specimens for cultivation, capsule staining, and PCR; specimens should also be subjected to antigen detection testing, if available.

Specimens from Animals Suspected To Have Died of Anthrax

Although the organism is rapidly destroyed by putrefaction in the intact carcass, the carcass of an animal that died of anthrax still generally yields positive cultures from appropriate specimens, such as blood from a peripheral vein, or from snips of tissue such as from the tip of an ear, as sporulation will still occur in some tissues; however, hemorrhagic exudate is

preferred to aspirated blood or tissue specimens for direct demonstration of the capsulated organism using Gram, Giemsa, or polychrome methylene blue (M'Fadyean) staining.

DIRECT EXAMINATION

The first examination of smears and cultures in many clinical laboratories is done with the Gram stain. In the past, it was regarded as of limited value in anthrax diagnosis, because the capsule is not seen. Notwithstanding this, if large numbers of gram-positive bacteria are observed in a patient's blood at death, then *B. anthracis* should be suspected. As in the 2001 bioterrorism incident in the United States, such preparations can be very useful (Fig. 1a). In other circumstances and in animals in particular, the blood or other specimen may not be collected soon after death and before putrefactive organisms appear; *B. anthracis* may then be indistinguishable without the use of the proper capsule stain.

The M'Fadyean polychrome methylene blue staining test dates from 1903 and has proved a remarkably successful rapid diagnostic test over the decades. Satisfactory polychrome methylene blue is now hard to obtain, but the slow, natural

ripening process that produces it may be hastened by adding 1% K_2CO_3 to Loeffler's alkaline methylene blue. For putrefied specimens, Giemsa-type stains may be better. For more information on capsule staining, see reference 140.

In addition to culturing *B. anthracis*, there are molecular and antigen-based detection methods available for direct detection in clinical and environmental samples. These detection methods may provide additional information. Several methods, including a *B. anthracis*-specific Laboratory Response Network (LRN) PCR assay, IHC assays, and serology, were useful for confirmation of cases during the 2001 bioterrorism-associated outbreak and more recent cases associated with drum making using *B. anthracis*-contaminated hides (23, 66, 112, 113, 122).

The IHC assay, as performed at the CDC, uses the same antibodies as the direct fluorescent-antibody (DFA) assay (specific to cell wall antigen or the capsule) to detect *B. anthracis* in formalin-fixed, paraffin-embedded tissues. This method was particularly useful in the diagnosis of cutaneous cases during the 2001 bioterrorism-associated outbreak. Skin biopsy samples from cutaneous lesions from 8 of 10 patients were positive for both the capsule and cell wall antigens (122). The most widely used and available detection

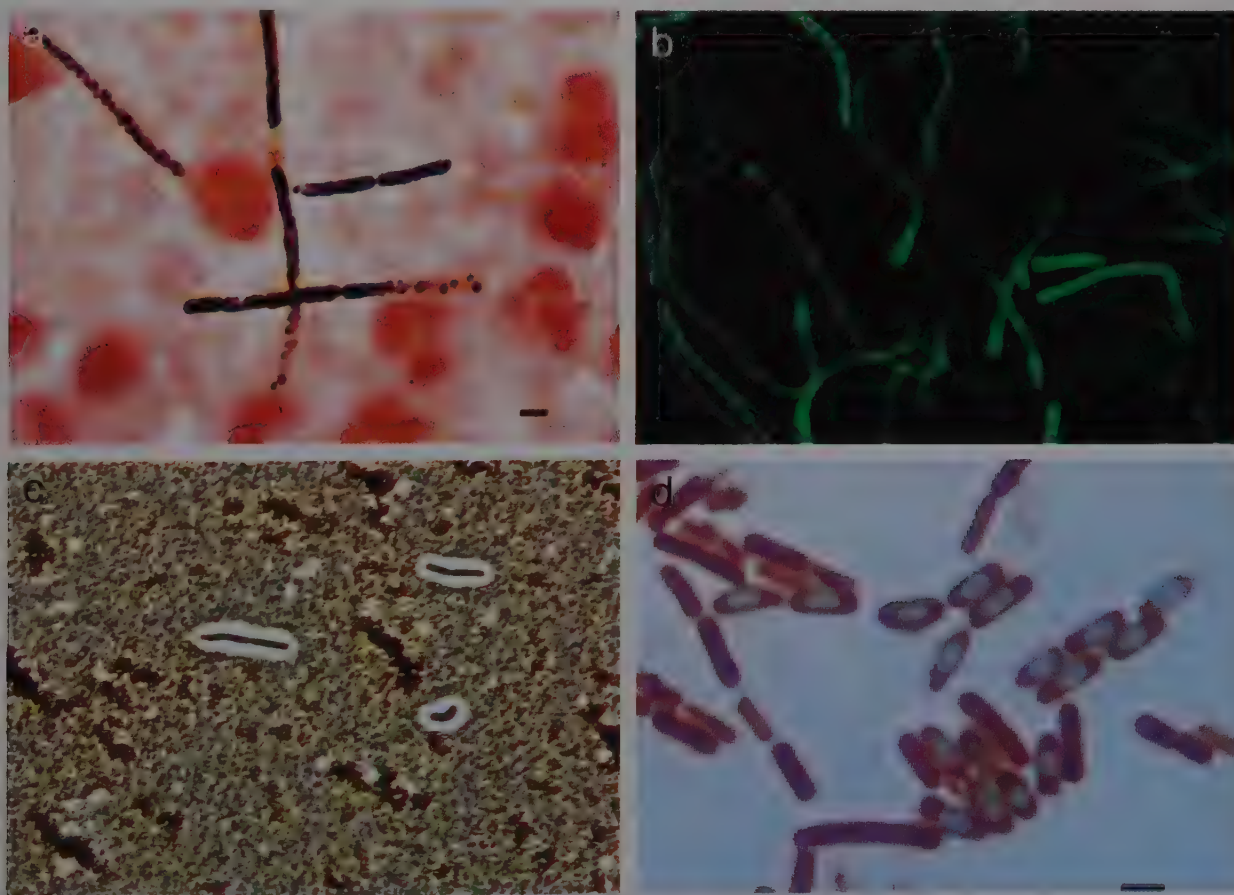


FIGURE 1 (a) Gram stain of *B. anthracis*, associated with a bioterrorism attack, showing gram-positive rods in peripheral blood buffy coat following admission of patient; bar marker represents 3 μm . (Courtesy of H. Masur.) (b) DFA-stained preparation of *B. anthracis* using an antibody specific for the poly- γ -D-glutamic acid capsule. (c) India ink stain of *B. anthracis* incubated in horse blood. Clear zones surrounding the rods are due to the exclusion of the India ink by the capsule. (d) Spore-stained preparation of *Bacillus cereus* sporangia, viewed by bright-field microscopy. Spores are stained green, and vegetative cells are counterstained red. Bar marker represents 2 μm . (Photograph kindly provided by M. Rodríguez-Díaz.)

method in the U.S. public health system is the LRN PCR (66). In addition to being widely used in 2001, this assay and the IHC assay were used in 2007 to diagnose two cutaneous anthrax cases in a drum maker and his child (23).

At the CDC, a positive PCR result on any clinical specimen from a patient collected from a normally sterile site (such as blood or CSF) or a lesion of other affected tissue (skin, pulmonary, reticuloendothelial, or gastrointestinal) is regarded as a supportive or presumptive diagnostic test. It is considered sufficient to provide a probable diagnosis but is not confirmatory in itself. The principal reason for such stringent guidelines on the use of PCR approaches and the value of their results towards providing a confirmatory diagnosis is based on the possibility that environmental contamination of a non-anthrax-related lesion could result in a positive result; this is especially the case with the use of some previously published PCR primers for capsule and chromosomal genes that can produce false positives with reactions to soil microbiota. This is quite similar to the recommendations that are included in the 2008 WHO guidelines (140), in which PCR can be used for identification of an isolate but is not recommended for testing of specimens.

There are numerous reports on the use of alternative technologies for the detection of *B. anthracis*, such as mass spectrometry, flow cytometry, time-resolved fluorescent assays, high-performance liquid chromatography, and even the use of engineered B cells to detect *B. anthracis* (5, 69, 115, 149, 152). Two recent studies focused on detecting the anthrax toxins, which are highly expressed during infection, in specimens instead of directly detecting the bacilli (15, 133). One reported on an immunoassay using highly fluorescent europium nanoparticles to detect protective antigen (PA), while the other reported detection of lethal factor (LF) by taking advantage of its metalloprotease activity, which cleaves specific peptides. The LF protein was captured by monoclonal antibodies and then incubated with a synthetic peptide substrate whose cleavage products were detected by matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry (15). These and other novel approaches may allow for detection of *B. anthracis* infection earlier in the course of the disease and thus allow for more successful treatment.

ISOLATION

Isolation from Human Specimens

Fresh specimens from patients should be inoculated onto plates of blood agar in the normal way. Enrichment procedures are generally inappropriate for isolations from clinical specimens. However, when seeking *B. cereus* in stools several days after a food poisoning episode, nutrient or tryptic soy broth with polymyxin (100,000 U/liter) may be added to a heat-treated specimen. Heat treatments at 70°C for 30 min or 80°C for 10 min are widely used for aerobic endospore formers in general, but 62 to 65°C for 15 to 20 min is more suitable for *B. anthracis*; temperatures above 70°C are not recommended for this species (142). There is no effective enrichment method for *B. anthracis* in old animal specimens or environmental samples, and isolation from these is best attempted using polymyxin-lysozyme EDTA-thallous acetate (PLET) agar (80; see also chapter 17). Aliquots (0.1 ml) of the undiluted suspension and 1:10 and 1:100 dilutions of a heat-treated suspension of the specimen are spread across PLET plates, which are read after incubation for 36 to 40 h at 37°C. Creamy white, domed, circular colonies, 1 to 3 mm, are subcultured onto (i) blood agar plates to

test for gamma phage and for hemolysis and (ii) directly or subsequently in blood to look for capsule production using either the M'Fadyean stain or India ink negative stain (the latter is less reliable, because the ink coagulates the blood and makes interpretation difficult); 2.5 ml of blood (preferably defibrinated horse blood, but horse or fetal calf serum is satisfactory) is inoculated with a pinhead quantity of growth from the suspect colony, incubated statically for 6 to 18 h at 37°C, and then stained. A differential/selective chromogenic medium is marketed by R&F Laboratories, Downers Grove, IL (R&F *Anthraxis* chromogenic agar), and it has undergone some independent evaluation (72), but another study (97) found PLET to be more sensitive and selective.

Several media have been designed for the isolation, identification, and enumeration of *B. cereus* organisms. They exploit the organism's egg yolk reaction (phospholipase C) positivity and acid-from-mannitol negativity; pyruvate and polymyxin may be included for selectivity. Three satisfactory formulations are MEYP or MYP (mannitol, egg yolk, polymyxin B agar; MEYP, Oxoid, Basingstoke, United Kingdom; MYP, Difco, BD, Franklin Lakes, NJ), PEMBA (polymyxin B, egg yolk, mannitol, bromthymol blue agar; Oxoid), and BCM (*Bacillus cereus* medium; LabM, Bury, United Kingdom) (see chapter 17). There are more recent formulations that reveal phospholipase C positivity using specific chromogenic substrates rather than natural egg yolk: *Bacillus cereus* group plating medium (Biosynth Chemistry and Biology, Staad, Switzerland, also available as *Cereus*-Ident-Agar from Heipha, Eppelheim, Germany) and *Bacillus cereus*/*Bacillus thuringiensis* chromogenic plating medium (R&F Laboratories).

There are no selective media for other *Bacillus* species, but spores can be selected for by heat treating part of the specimen as described above; however, this is not appropriate for fresh clinical specimens, where spores are usually sparse or absent. Vegetative cells of both sporeformers and non-sporeformers are killed by heat treatment, but the heat-resistant spores not only survive but also may be heat shocked into subsequent germination; another part of the specimen is cultivated without heat treatment in case spores are very heat sensitive or absent.

In specimens submitted for food poisoning investigations, or for isolation of *B. anthracis* from old carcasses, animal products, or environmental specimens, the organisms will mostly be present as spores. Heat treatment, as described above, will both heat shock the spores and effectively destroy non-spore-forming contaminants. A variety of approaches are used to process dry or solid samples prior to heat treatment. Direct plate cultures are then made on blood, nutrient, or selective agars, as appropriate, by spreading up to 0.1-ml volumes from undiluted sample and 10- and 100-fold dilutions of the treated sample.

Isolation from Animals Suspected To Have Died of Anthrax

Anthrax should be considered as the possible cause of death in herbivorous animals that have died suddenly and unexpectedly, especially if hemorrhage from the nose, mouth, or anus has occurred, and if death has taken place at a site with a history of anthrax—perhaps even several decades before. PCR-based approaches are becoming more widely used for direct detection of *B. anthracis* in veterinary specimens.

Carcasses 1 to 2 Days Old

Due to the nonclotting nature of blood in animals that have died of anthrax infection, it is usually possible to

aspirate a few drops of blood from a peripheral vein for (i) a M'Fadyean-stained smear and (ii) direct plate culture on blood agar.

In pigs, the enormous terminal bacteremia seen in herbivores may not develop, and the capsulated rods may not be visible in blood smears. When cervical edema is present, smears and cultures should be made of fluid aspirated from the enlarged mandibular and suprathyroid lymph nodes. Intestinal anthrax of pigs may be obvious only at necropsy, when rods are usually visible in stained smears made from mesenteric lymph nodes.

Older Putrefying Carcasses

The organism may not be visible in smears 2 to 3 days following death, as it competes poorly with putrefactive organisms; culture is then necessary for diagnostic confirmation. Sections of tissue, or any blood-stained material, should be collected, and spleen or lymph node specimens should be taken if the animal has been opened. With putrefied and very old carcasses, swabs of the nostrils, nasal turbinates, and eye sockets are likely to yield *B. anthracis*, but the best specimens may be samples of contaminated soil taken from beneath the head and tail.

Isolation of *B. anthracis* from Bioterrorism-Related Specimens

In 1999, an LRN was established in the United States by the CDC in partnership with the Association of Public Health Laboratories, Federal Bureau of Investigation, and Department of Defense to provide the public health laboratory response to acts of bioterrorism (103). This network links local (sentinel) laboratories to laboratories with more specialized testing and increased biosafety capacity at the state (reference) and federal (national) levels. There are reference level laboratories in all 50 states able to detect agents, including *B. anthracis*, rapidly. State public health laboratories are part of the LRN and are able to provide guidance, or the LRN can be accessed using the Internet (<http://www.bt.cdc.gov/lrn/>). If unable to reach the state public health laboratory during nonbusiness hours, LRN consultations may be requested by calling the CDC Emergency Operations Center at 770-488-7100. State and territorial public health laboratory contact information and sentinel laboratory guidelines are also available on the American Society for Microbiology website at <http://www.asm.org>. For general questions there is a 24-h hotline number, 800-CDC-INFO (800-232-4636), and an e-mail address, cdcinfo@cdc.gov. Although initially limited to the United States, there are now over 150 national and international locations, including laboratories within Canada, Australia, Germany (U.S. military base), Japan (U.S. military base), South Korea (U.S. military base), and the United Kingdom, capable of providing a rapid response to acts of biological terrorism, emerging infectious diseases, and other public health threats and emergencies.

Isolation of *B. anthracis* from Environmental and Animal Product Specimens

Tests for the presence of *B. anthracis* may be requested for diverse specimens, such as animal products (e.g., wool, hides, hair, and bonemeal) from regions of endemicity, soil or other materials from old burial sites or tannery or laboratory sites due for redevelopment, or other environmental materials associated with outbreaks (e.g., sewage sludge). Detection in such specimens may mean searching for rather few spores of *B. anthracis* among those of many other species,

especially other members of the *B. cereus* group. Some environmental specimens may contain substances that inhibit germination and growth of *B. anthracis* (140). At present, there is no enrichment method for *B. anthracis*, and culture by the selective agar techniques described above is the best approach. PCR-based methods are being used increasingly for the direct detection of *B. anthracis* in clinical and environmental samples (125), but it is still advisable to confirm positive results by conventional methods.

IDENTIFICATION

Remember that these organisms do not always stain gram positive; some are gram variable, gram positivity is readily lost in older cultures, and some species or strains are frankly gram negative. Before attempting to identify to the species level, it is important to establish that the isolate really is an aerobic endospore former and that other inclusions are not being mistaken for spores. Phase contrast (at a magnification of $\times 1,000$) should be used if available, as it is superior to spore staining and more convenient. Spores are larger, more phase bright, and more regular in shape, size, and position than other kinds of inclusions such as polyhydroxybutyrate (PHB) granules (Fig. 2d), and sporangial appearance is valuable in identification (Fig. 2). For spore staining, flood a heat-fixed smear with 10% aqueous malachite green for up to 45 min (without heating), followed by washing and counterstaining with 0.5% aqueous safranin for 30 s; spores are green within pink-red cells at a magnification of $\times 1,000$ (Fig. 1d). A Gram-stained smear showing cells with unstained areas suggestive of spores can be stripped of oil with acetone-alcohol, washed, and then stained for spores.

Isolates have often been submitted to reference laboratories as *Bacillus* species because they were large, aerobic gram-positive rods, even though sporulation had not been observed, or because PHB granules or other storage inclusions had been mistaken for spores. Members of the *B. cereus* group and *B. megaterium* produce large amounts of storage material when grown on carbohydrate media, but on routine media this vacuolate or foamy appearance is rarely sufficiently pronounced to cause confusion.

Bacillus and related genera contain facultative anaerobes as well as strict aerobes, which can be a valuable characteristic in identification. For example, *B. licheniformis* and *B. subtilis*, which have very similar colonial (Fig. 3j) and microscopic (Fig. 2e) morphologies, are facultatively anaerobic and strictly aerobic, respectively. Likewise, the two large-celled species *B. cereus* and *B. megaterium* (Fig. 2b and d) are facultatively anaerobic and strictly aerobic, respectively.

The most widely used diagnostic schemes use traditional phenotypic tests, or miniaturized tests of the API 20E and 50CHB kits used together (90) (bioMérieux, Marcy l'Etoile, France). The API 20E and 50CHB kits can be used for the presumptive distinction of *B. anthracis* from other members of the *B. cereus* group within 48 h. bioMérieux also offers identification cards for *Bacillus* and related genera for the VITEK and VITEK Compact automated identification systems. As many new species have been proposed since these schemes were established, updated API and VITEK databases have been prepared. Biolog Inc. (Hayward, CA) also offers a *Bacillus* database. The effectiveness of such kits can vary with the genera and species of aerobic endospore formers concerned, but they are improving with continuing development and enlarged databases. The many proposals for new species, often on the basis of single isolates, make the satisfactory expansion of such databases problematic; for a database to be

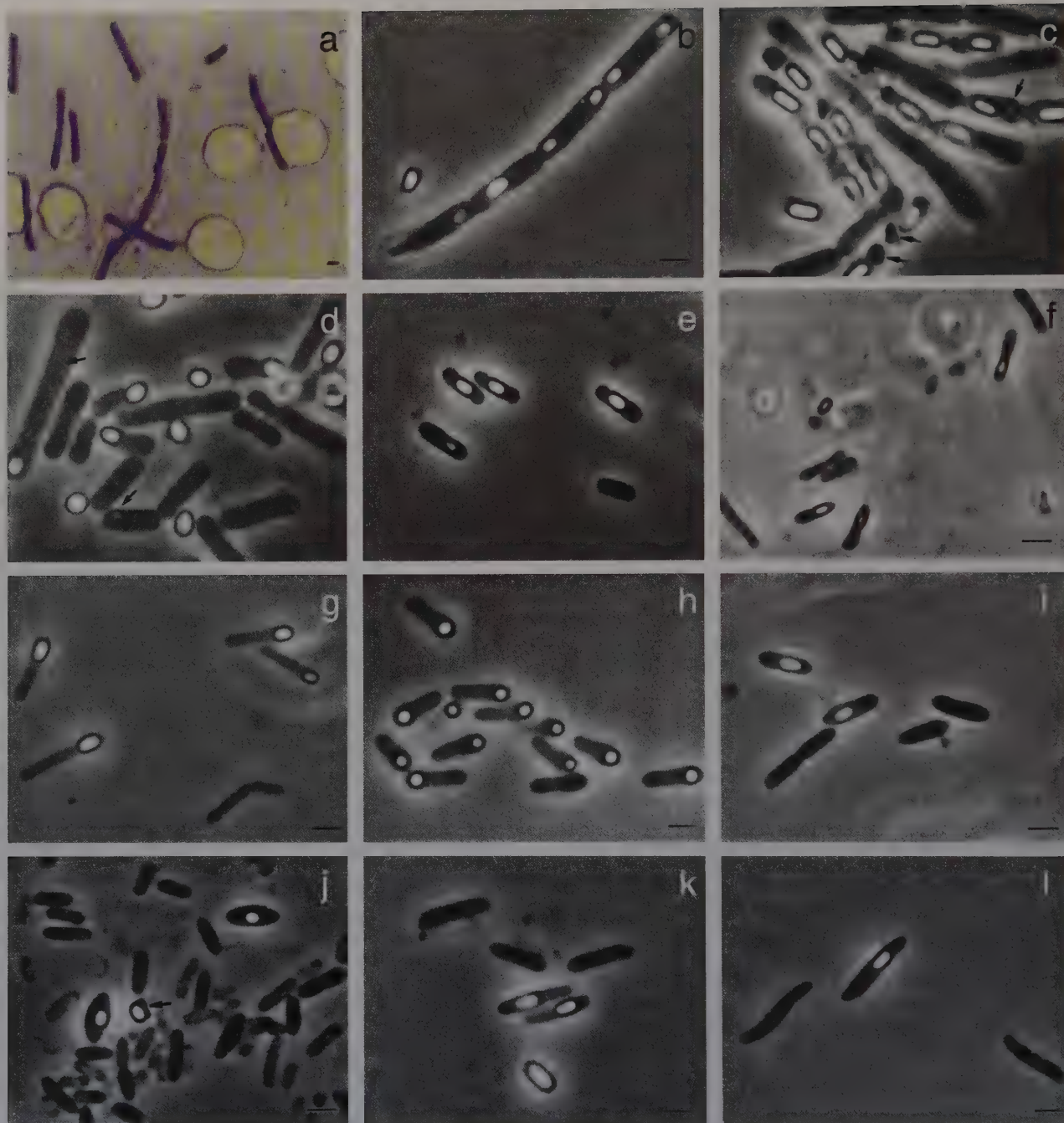


FIGURE 2 Photomicrographs of endospore-forming bacteria viewed by bright-field microscopy (a) and phase-contrast microscopy (b to l). Bar markers represent 2 μm . (a) *B. anthracis*, M'Fadyean stain showing capsule rods in guinea pig blood smear; (b) *B. cereus*, broad cells with ellipsoidal, subterminal spores, not swelling the sporangia; (c) *B. thuringiensis*, broad cells with ellipsoidal, subterminal spores, not swelling the sporangia, and showing parasporal crystals of insecticidal toxin (arrows); (d) *B. megaterium*, broad cells with ellipsoidal and spherical, subterminal and terminal spores, not swelling the sporangia, and showing PHB inclusions (arrows); (e) *B. subtilis*, ellipsoidal, central and subterminal spores, not swelling the sporangia; (f) *B. pumilus*, slender cells with cylindrical, subterminal spores, not swelling the sporangia; (g) *B. circulans*, ellipsoidal, subterminal spores, swelling the sporangia; (h) *Lysinibacillus sphaericus*, spherical, terminal spores, swelling the sporangia; (i) *Brevibacillus brevis*, ellipsoidal, subterminal spores, one swelling its sporangium slightly; (j) *Brevibacillus laterosporus*, ellipsoidal, central spores with thickened rims on one side (arrow), swelling the sporangia; (k) *Paenibacillus polymyxa*, ellipsoidal, paracentral to subterminal spores, swelling the sporangia slightly; (l) *Paenibacillus alvei*, cells with tapered ends, ellipsoidal, paracentral to subterminal spores, not swelling the sporangium.

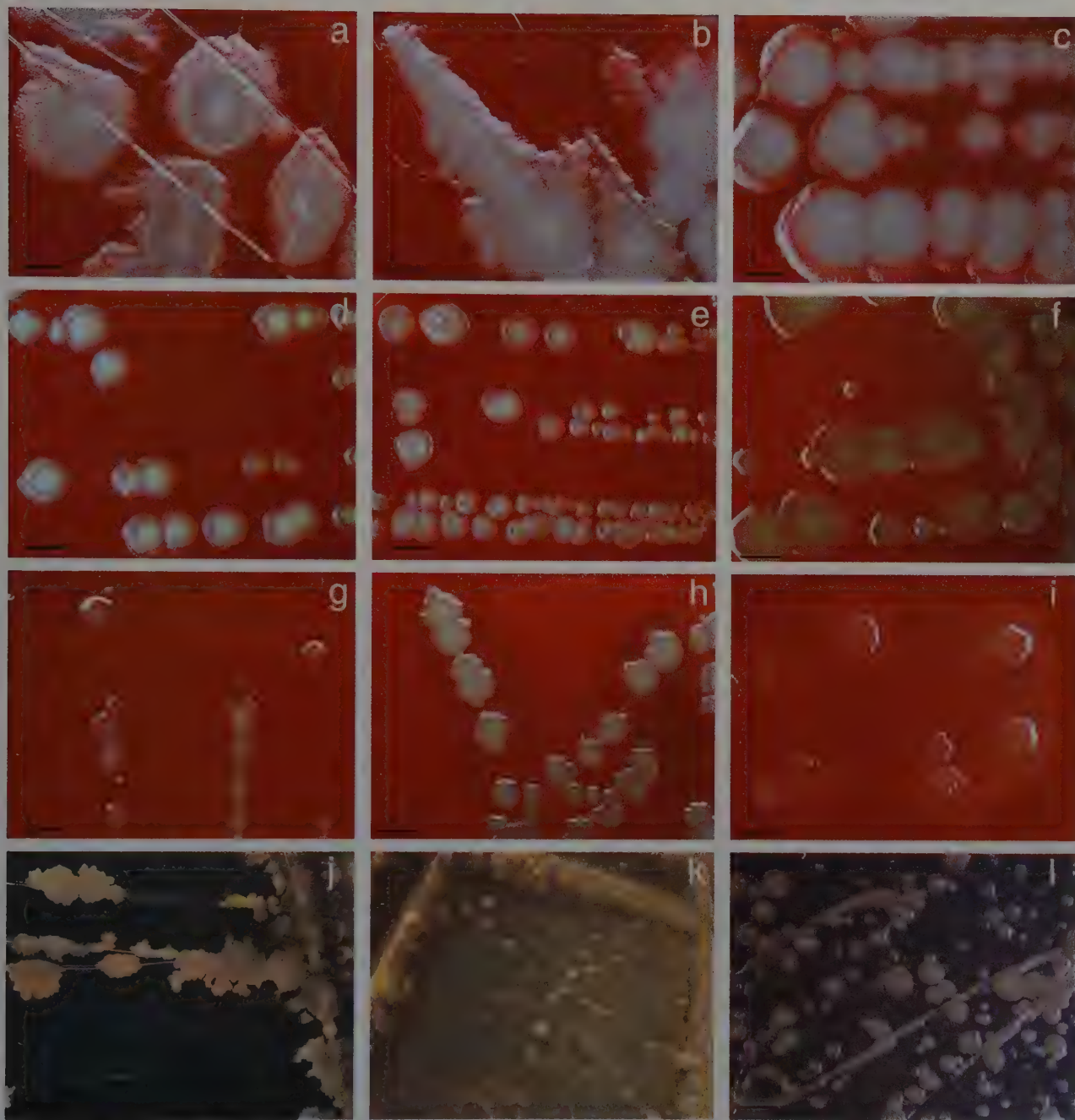


FIGURE 3 Colonies of endospore-forming bacteria on blood agar (a to i) and nutrient agar (j to l) after 24 to 36 h at 37°C. Bar markers represent 2 mm. (a) *B. anthracis*; (b) *B. cereus*; (c) *B. thuringiensis*; (d) *B. megaterium*; (e) *B. pumilus*; (f) *Lysinibacillus sphaericus*; (g) *Brevibacillus brevis*; (h) *Brevibacillus laterosporus*; (i) *Paenibacillus polymyxa*; (j) *B. subtilis*; (k) *B. circulans*; (l) *Paenibacillus alvei*.

effective in identifying a particular species, its entry for that species needs to reflect the characterization of at least 10 authentic strains from a range of sources, but this requirement can be very difficult, even impossible, to fulfill. It is stressed that the use of these kits should always be preceded by the basic characterization tests described below.

Other approaches include chemotaxonomic fingerprinting by fatty acid methyl ester profiling, pyrolysis mass spectrometry, and Fourier transform infrared spectroscopy. All these approaches have been successfully applied either across the genera or to small groups. As with genotypic profiling

methods, large databases of authentic strains are necessary; some of these are commercially available, such as the Microbial Identification System software (Microbial ID Inc., Newark, DE) database for fatty acid methyl ester analysis.

For diagnostic purposes, the aerobic endospore formers comprise two groups: the reactive ones, which give positive results in various routine biochemical tests and which are therefore easier to identify, and the nonreactive ones, which give few, if any, positive results in such tests. Nonreactive isolates tend to dominate the identification requests sent to reference laboratories. Table 1 shows reactions for some

TABLE 1 Characters for differentiating some species of *Bacillus*, *Geobacillus*, *Paenibacillus*, and *Virgibacillus*^a

Character ^b	<i>Bacillus</i>								
	<i>B. subtilis</i> group				<i>B. cereus</i> group				<i>B. megaterium</i>
	<i>B. subtilis</i>	<i>B. amyloliquefaciens</i>	<i>B. licheniformis</i>	<i>B. pumilus</i>	<i>B. cereus</i> ^c	<i>B. anthracis</i>	<i>B. thuringiensis</i>	<i>B. mycoides</i>	
Rod mean diam. (μm)	0.8	0.8	0.8	0.7	1.2	1.2	1.2	1.2	1.4
Chains of cells	(-)	(+)	(+)	-	+	+	+	+	+
Motility	+	+	+	+	+	-	+	-	+
Sporangia ^d									
Spore shape	E	E	E (C)	C, E	E (C) [E]	E	E (C)	E	E, S
Spore position	S, C	S, C	S, C	S, C	S, C	S	S	S (C)	S, C
Sporangium swollen	-	-	-	-	-	-	-	-	-
Parasporal crystals	-	-	-	-	-	-	+	-	-
Anaerobic growth	-	-	+	-	+	+	+	+	-
Growth at:									
50°C	v	v	+	v	-	-	-	-	v
65°C	-	-	-	-	-	-	-	-	-
Egg yolk reaction	-	-	-	-	+	+	+	+	-
Casein hydrolysis	+	+	+	+	+	+	+	+	+
Starch hydrolysis	+	+	+	-	+	+	+	+	+
Arginine dihydrolase	-	-	(+)	-	v [(-)]	-	+	v	-
Indole production	-	-	-	-	-	-	-	-	-
Gelatin hydrolysis	+	+	+	+	+	+	+	+	+
Nitrate reduction	+	+	+	-	(+) [v]	+	+	(+)	v
Gas from carbohydrates	-	-	-	-	-	-	-	-	-
Acid from:									
D-Arabinose	-	-	-	-	-	-	-	-	-
Glycerol	+	+	+	+	+	-	+	+	+
Glycogen	+	+	+	-	+	+	+	+	+
Inulin	(+)	-	v	-	-	-	-	-	+
Mannitol	+	+	+	+	-	-	-	-	+
Salicin	+	+	+	+	+	-	(+)	(+)	+
D-Trehalose	+	+	+	+	+	+	+	+	+

^a Symbols and abbreviations: +, ≥85% positive; +/w, positive or weakly positive; w, weakly positive; (+), 75 to 84% positive; v, variable (26 to 74% positive); (-), 16 to 25% positive; -, 0 to 15% positive; -/w, negative or weakly positive.

^b Arginine dihydrolase, indole production, gelatin hydrolysis, and nitrate reduction reactions were determined using tests in the API 20E strip (bioMérieux). Acid from carbohydrate reactions was determined using the API 50CHB System (bioMérieux).

species belonging to the former group, and the phenotypic test scheme outlined above may be used in conjunction with it.

Identification and Detection of *B. anthracis*

It is generally easy to distinguish virulent *B. anthracis* from other members of the *B. cereus* group. *B. anthracis* isolates are characterized by typical microscopic appearance (Fig. 1b and 2a) and colonial morphology (Fig. 3a): colonies are white or gray, nonhemolytic or only weakly hemolytic, susceptible to the diagnostic gamma phage (inquiries about gamma phage should be addressed to the Diagnostics Systems Division, USAMRIID, Fort Detrick, Frederick, MD), generally susceptible to penicillin, nonmotile, and able to produce the characteristic capsule as shown by M'Fadyean staining (Fig. 2a) or India ink staining (Fig. 1b). As an alternative to culture in blood, the capsule of virulent *B. anthracis* can be demonstrated on nutrient agar containing 0.7% sodium bicarbonate, incubated overnight under 5 to 7% CO₂ (candle jars perform well). Colonies of the capsulated organism appear mucoid, and the capsule can be visualized by M'Fadyean or India ink staining of smears or by DFA staining (Fig. 1c; see below).

In addition to phenotypic analysis, molecular and antigenic detection assays are available for the rapid identification of *B. anthracis*. The LRN PCR (restricted to LRN laboratories; see above) targets three distinct loci on the *B. anthracis* chromosome, pXO1 virulence plasmid, and pXO2 virulence plasmid. Using several loci increases specificity and allows for the detection of avirulent strains (lacking pXO1 or pXO2). The anthrax toxin genes (*pagA*, *lef*, and *cya*) are located on pXO1, while the genes required for capsule biosynthesis (*capBCA*) are located on pXO2. Isolates lacking pXO2 or both plasmids are mostly found in the environment and are frequently mistaken for *B. cereus*, due to the lack of a capsule, and discarded. These genes have been widely used as *B. anthracis*-specific gene targets; however, there have been recent reports of these genes in species other than *B. anthracis* (8, 55, 67). Recently several laboratories have developed specific PCR assays for *B. anthracis* that target chromosomal genes such as *rpoB*, *gyrA*, and *plcR* (39, 70, 111).

A two-component DFA assay has been and is currently used to identify encapsulated vegetative cells of *B. anthracis* (33, 45). This assay uses two different monoclonal

<i>Bacillus</i>				<i>Geobacillus</i>		<i>Paenibacillus</i>				<i>Virgibacillus pantothenicus</i>
<i>B. circulans</i> group										
<i>B. circulans</i>	<i>B. firmus</i>	<i>B. lentus</i>	<i>B. coagulans</i>	<i>G. stearothermophilus</i>	<i>G. thermodenitrificans</i>	<i>P. polymyxa</i>	<i>P. alvei</i>	<i>P. macerans</i>	<i>P. validus</i>	
0.8	0.8	0.8	0.8	0.9	0.8	0.9	0.8	0.7	0.8	0.6
—	(+)	(+)	v	v	v	—	(—)	—	—	+
+	+	+	+	+	+	+	+	+	+	+
E	E (C)	E	E (S)	E	E	E	E	E	E	E, S
S, T	S, C	S, C	S, C, T	S, T	S, T	S (C)	S, C	S (T)	S, T	T (S)
+	v	v	+	v	—	+	+	+	+	+
—	—	—	—	—	—	—	—	—	—	—
+	+	—	+	—/w	+	+	+	+	—	+
+	v	—	+	+	+	—	—	v	+	v
—	—	—	—	+	+	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—
w	w	—	—	+/w	—	+	+	—	—	+
+	+	+	+	+	+/w	+	+	+	+	+
—	—	—	v	—	—	—	—	—	—	(—)
—	—	—	—	—	—	—	+	—	—	—
v	+	—	v	+	v	+	+	v	—	+
v	+	+	v	v	v	+	—	v	v	v
—	—	—	—	—	—	+	—	+	—	—
—	—	—	—	—	—	—	—	+	—	+
+	v	—	+	w	—	+	+	+	+	+
+	v	—	—	+	—	+	v	+	v	—
+	—	—	—	v	—	+	—	+	v	—
+	+	+/w	—	—	w	+	—	+	+	—
+	—	+/w	v	—	v	+	v	+	—	+
+	v	+	+	+	+	+	v	+	+	+

^c Reactions shown in brackets are for the biotype isolated particularly in connection with outbreaks of emetic-type food poisoning and for strains of serovars 1, 3, 5, and 8, which are commonly associated with such outbreaks.

^d Spore shape: C, cylindrical; E, ellipsoidal; S, spherical. Spore position: C, central or paracentral; S, subterminal; T, terminal. The commonest shapes and positions are listed first, and those shown in parentheses are infrequently observed.

antibodies specific for a *B. anthracis* cell wall antigen and the *B. anthracis* capsule (Fig. 1c). Neither antigen is 100% specific for *B. anthracis*; however, only *B. anthracis* has been found to be positive for both antigens, and thus, the assay is 100% specific when both cell wall and capsule components are used together. It was heavily used at the CDC during the 2001 bioterrorism-associated outbreak for the rapid (<4-h) identification of isolates (33).

Tetracore Inc. (Gaithersburg, MD) has produced a rapid (yielding a result within 15 min) immunochromatographic test (RedLine Alert) utilizing an antibody specific for one of the *B. anthracis* S-layer proteins. This assay has been approved by the Food and Drug Administration (FDA) for use on nonhemolytic *Bacillus* species colonies cultured on sheep blood agar plates. Manufacturer's data suggest that the test was 98.6% sensitive when tested on 145 *B. anthracis* isolates and 45 nonhemolytic, non-*B. anthracis* isolates; however, such identification of *B. anthracis* is only considered presumptive, and this test should not be used as a stand-alone test.

An immunochromatographic field assay has been developed by the U.S. Naval Medical Research Center (NMRC),

Silver Spring, MD, to detect PA in samples of blood or tissue exudates (*Bacillus anthracis* immunochromatographic field assay). Inquiries from veterinary and public health authorities should be directed to the NMRC by calling 301-319-7409 or in writing to the Naval Medical Research Center, 503 Robert Grant Ave., Silver Spring, MD 20910 (J. Czarnecki, NMRC, personal communication, 2007). The assay has been used to detect *B. anthracis* in animals, even several days after death. The assay has a high sensitivity for the detection of *B. anthracis* in an infected animal and has a high specificity (regarded as 100%; 95% confidence interval, 98.5 to 100%) for detection of the organism in cattle (104). Among 10 recently vaccinated bovines in one study, the assay yielded no false-positive reactions.

Identification of *Bacillus cereus* and *B. thuringiensis*

Colonies of *B. cereus* and relatives are very variable but readily recognized (Fig. 3a to c): they are characteristically large (2 to 7 mm in diameter) and vary in shape from circular to irregular, with entire to undulate, crenate or fimbriate edges; they have matte or granular textures. Smooth and moist colonies are not uncommon, however.

The optimum growth temperature is about 37°C, with minima and maxima of 15 to 20°C and 40 to 45°C, respectively. Although colonies of *B. anthracis* and *B. cereus* can be similar in appearance, those of the former are generally smaller and nonhemolytic, may show more spiking or tailing along the lines of inoculation streaks, and are very tenacious compared with the usually more butyrous consistency of *B. cereus* and *B. thuringiensis* colonies, so that they may be pulled into standing peaks with a loop. *Bacillus mycoides* produces characteristic rhizoid or hairy-looking, adherent colonies which readily cover the whole agar surface.

The key characteristics for recognizing and distinguishing the *B. cereus* group are colonial morphology (Fig. 3a to c); large cells often in chains, producing ellipsoidal spores not swelling the sporangia (Fig. 2b and c), usually within 48 h and often apparent after 24 h; facultative anaerobes; and positive egg yolk reaction (i.e., lecithinase). Negative or very weak hemolysis and lack of motility distinguish *B. anthracis* and *B. mycoides* from *B. cereus* and *B. thuringiensis*. *Bacillus cereus*, *B. mycoides*, *B. thuringiensis*, and, to a lesser extent, *B. anthracis* synthesize lecithinases, forming opaque zones of precipitation around colonies on egg yolk agar as the colonies grow (i.e., usually after overnight or perhaps 24 h of incubation). Recognition of *B. thuringiensis* is largely dependent on observation of its cuboid or diamond-shaped parasporal crystals in sporulated cultures (after 2 to 5 days) by phase-contrast microscopy (Fig. 2c), or by staining with malachite green counterstained with carbol fuchsin or safranin.

Toxin Detection

The enterotoxin complex responsible for the diarrheal type of *B. cereus* food poisoning has been increasingly well characterized (128). Two commercial kits are available for its detection in foods and feces, the Oxoid BCET-RPLA (Oxoid Ltd.) and the TECRA VIA (TECRA Diagnostics, Roseville, New South Wales, Australia). However, these kits detect different antigens, and there is some controversy about their reliabilities. Other assays, based on tissue culture, have also been developed. The emetic toxin of *B. cereus*, cereulide, has been identified as a dodecadepsipeptide, and it may be nonspecifically assayed in food extracts or culture filtrates using HEp-2 cells (46), boar semen (4), and rat mitochondria (75). Specific detection requires high-performance liquid chromatography-mass spectrometry (59), and the synthetic apparatus may be detected by real-time PCR (49).

Other Species

Other species show a very wide range of colonial morphologies, both within and between species after 24 to 48 h (Fig. 3). They vary from moist and glossy (Fig. 3f and i) through granular to wrinkled (Fig. 3e); shape varies from round to irregular (Fig. 3d to i), sometimes spreading (Fig. 3k and l), with entire through undulate or crenate to fimbriate edges (Fig. 3d to j). Sizes range from 1 to 5 mm; color commonly ranges from buff or creamy gray to off-white, but some strains may produce orange pigment. Hemolysis may be absent, slight or marked, partial, or complete (Fig. 3h); elevations range from effuse through raised to convex. Consistency is usually butyrous, but mucoid and dry, adherent colonies are not uncommon. Despite this diversity, *Bacillus* colonies are not generally difficult to recognize, and some species have characteristic yet seemingly infinitely variable colonial morphologies, as does the *B. cereus* group (Fig. 3a to c).

Bacillus subtilis and *B. licheniformis* produce similar colonies which are exceptionally variable in appearance and often appear to be mixed cultures (Fig. 3j); colonies are irregular in shape and of moderate (2 to 4 mm) diameter and range from moist and butyrous or mucoid, with margins varying from undulate to fimbriate through membranous with an underlying mucoid matrix, with or without mucoid beading at the surface, to rough and crusty as they dry. The "licheniform" colonies of *B. licheniformis* tend to be quite adherent.

Rotating and migrating microcolonies, which may show spreading growth (Fig. 3k), have been observed macroscopically in about 13% of strains received as *B. circulans*, but this very heterogeneous species continues to undergo radical taxonomic revision, and such spreading strains are now assigned to *Paenibacillus* species (Fig. 3l).

Other species that have been encountered in the clinical laboratory include *B. coagulans*, *B. megaterium*, *B. pumilus*, and *B. sphaericus*; *B. brevis* and *B. laterosporus* (now both in *Brevibacillus*); and *B. macerans* and *B. polymyxa* (now both in *Paenibacillus*), and they do not produce particularly distinctive growth (Fig. 3d to i).

Microscopic morphologies, particularly of sporangia (Fig. 2), are much more helpful for distinguishing between species. Vegetative cells are usually round ended, but those of *P. alvei* may be tapered (Fig. 2l). The large cells of *B. megaterium* may accumulate PHB (Fig. 2d) and appear vacuolate or foamy when grown on glucose nutrient agar. Overall, cell widths vary from about 0.5 to 1.5 μ m and lengths from 1.5 to 8 μ m. Most strains of these species are motile. Spore shapes vary from cylindrical (Fig. 2f) through ellipsoidal (Fig. 2b, c to e, g, and i to l) to spherical (Fig. 2d and h); bean- or kidney-shaped, curved-cylindrical, and pear-shaped spores are also seen occasionally. Spores may be terminally (Fig. 2h), subterminally (Fig. 2b, c, f, g, and i), or centrally (Fig. 2e and j) positioned within sporangia and may distend them (Fig. 2g and k). Despite within-species and within-strain variation, sporangial morphologies tend to be characteristic of species and may allow tentative identification by the experienced worker. One species, *Brevibacillus laterosporus*, produces very distinctive ellipsoidal spores that have thickened rims on one side, so that they appear to be laterally displaced in the sporangia (Fig. 2j).

All these species are mesophilic and grow well between 30 and 37°C. Minimum growth temperatures lie mostly between 5 and 20°C and maxima mostly between 35 and 50°C. Strains of *B. coagulans* may show slight thermophily and grow up to 55 to 60°C.

TYPING SYSTEMS

Genotyping of *B. anthracis*

B. anthracis is a genetically monomorphic species and has been shown to be clonal by multilocus sequence typing (MLST). The first method described that could differentiate strains with any useful resolution was a multiple-locus variable-number tandem-repeat analysis (MLVA) assay targeting eight loci, MLVA-8 (76). This method was relied on during the 2001 bioterrorism-associated outbreak in the United States, which implicated the Ames strain, and continues to be useful today (76, 132). There are now multiple MLVA schemes available for the genotyping of *B. anthracis*, including expanded versions of the original MLVA-8. These include schemes which employ agarose electrophoresis, capillary electrophoresis, or mass spectrometry for

fragment analysis (76, 87, 89, 143). An online database, MLVAbank, is also now available at <http://minisatellites.upsud.fr/MLVAnet/>, which can accept data entry for 25 variable-number tandem repeats. There are also several reports on the analysis of single nucleotide repeats to differentiate very closely related isolates (77, 78, 87, 89, 131).

The sequencing of multiple *B. anthracis* genomes has also led to additional approaches to the genotyping of *B. anthracis*, such as the use of single nucleotide polymorphisms and DNA microarrays. Single nucleotide polymorphisms have been identified and used for the differentiation of *B. anthracis* lineages and for detection of specific strains such as the Ames strain (144, 145). This approach may continue to become more powerful as more genome data become available and are analyzed. Microarrays have also been used for strain characterization and comparisons (36, 153). However, they have not been widely used and seem to be less attractive now that newer *de novo* sequencing technologies have become more affordable and allow for the rapid generation of complete or almost complete genome sequence data. As technologies continue to improve and the costs decrease, the complete genomic sequence will increasingly be used for isolate characterization.

Genotyping of Other Species

The majority of work on molecular typing (i.e., genotyping) of *Bacillus* spp. has focused on members of the *B. cereus* group due to their clinical importance and the value of genotyping for molecular epidemiology. In the past, this group was differentiated into serovars based on flagellar antigen variations; however, this is not commonly used today and has largely been replaced by molecular methods. Numerous molecular methods have been attempted to some degree for the differentiation of *Bacillus* isolates. Recently, MLST, which compares the partial sequences of seven housekeeping genes to differentiate strains, has become the favored approach due to decreasing costs, portability of data, and availability of public databases on the World Wide Web. Several MLST schemes targeting different sets of genes have been reported and generally show similar clusters of isolates into three distinct clades (64, 81, 110, 126, 136). Pathogenic isolates are distributed throughout clades 1 and 2, while no clinical isolates have been identified as belonging in clade 3 (34, 68). With the exception of *B. anthracis* and emetic isolates of *B. cereus*, which are largely clonal, *B. cereus* group isolates are represented by a large number of distinct sequence types, and clustering does not correlate with classic microbiological species identification. Simpson's index of diversity (measure of the likelihood of two isolates from epidemiologically distinct events having the same sequence type) was calculated to be 0.989 (1.0 is absolute discrimination) in one study of clinical isolates, which suggests that in addition to being a powerful phylogenetic tool, it may be useful for molecular epidemiology (68). The most powerful aspect of MLST is the availability of online databases for several of the MLST schemes, which allows for worldwide access to view and deposit isolate data. The Priest scheme is available at <http://pubmlst.org/bcereus/>, and an optimized version of the Tourasse scheme, including a multischeme database (SuperCAT), is available at <http://mlstoslo.uio.no/> (110, 136, 137).

SEROLOGIC TESTS

Serologic assays for the detection of antibody response against the anthrax toxin protein, PA, have been used in combination with PCR or IHC assay results to confirm anthrax cases when culture failed. A quantitative human anti-PA

immunoglobulin G (IgG) enzyme-linked immunosorbent assay was performed at the CDC during the 2001 outbreak and was positive only with sera from individuals with anthrax or vaccinated with anthrax vaccine adsorbed (AVA; see "Vaccines" below) (112). An FDA-approved, qualitative kit (QuickELISA Anthrax-PA kit) from Immunetics (Boston, MA) is not currently available, but production could be initiated if needed for the detection of anti-PA IgG and IgM antibodies in human serum. Serologic assays aided in the effort to confirm cases in the 2001 attack, particularly cutaneous ones; however, the time to seroconversion after infection limits the usefulness of this approach, given the rapid diagnosis necessary for treatment and public health response.

The three protein components of anthrax toxin (PA, LF, and edema factor [EF]), and antibodies to them, can be used in enzyme immunoassay systems. For routine confirmation of anthrax infection or for monitoring response to anthrax vaccines, antibodies against PA alone appear to be satisfactory; they have proved useful for epidemiological investigations with humans and animals. In human anthrax, however, early treatment sometimes prevents development of a detectable rise in antibody titer (113). PA, LF, and EF are available commercially from List Biological Laboratories, Inc., Campbell, CA (<http://www.listlabs.com>).

In countries of the former USSR, a skin test utilizing anthraxin, a heat-stable extract from a noncapsulate strain of *B. anthracis* that has been licensed for human and animal use since 1962, is widely acclaimed for retrospective diagnosis (123). The delayed-type hypersensitivity is interpreted as indicating cell-mediated immunity to anthrax and can be used to evaluate the vaccine-induced immune status after periods of several years, as well as to diagnose anthrax retrospectively. Anthraxin does not contain highly specific anthrax antigens and relies on the fact that the only *Bacillus* species likely to proliferate within and throughout an animal is *B. anthracis*. This is also true of the Ascoli test, which, dating from 1911, must be one of the oldest antigen detection tests in microbiology. It is a precipitin test using hyperimmune serum raised to *B. anthracis* whole-cell antigen to provide rapid retrospective evidence of anthrax infection in an animal from which the material being tested was derived. The test is still in use in Eastern Europe and central Asia.

ANTIMICROBIAL SUSCEPTIBILITIES

Bacillus anthracis

Most strains of *B. anthracis* remain susceptible to penicillin (30, 102, 141). Of 25 genetically diverse isolates from around the world, three strains were resistant to penicillin but were negative for β -lactamase production. Most strains give variable susceptibility results for cephalosporins; in vitro results, even if susceptible, may not predict clinical efficacy, particularly for expanded- and broad-spectrum cephalosporins (30). In a study of 50 historical isolates from humans and animals and 15 clinical isolates from the 2001 bioterrorism attack in the United States, the majority of strains could be regarded as not susceptible to the broad-spectrum cephalosporin ceftriaxone, and 3 were resistant to penicillin (102). Tetracyclines, fluoroquinolones, and chloramphenicol are suitable for the treatment of patients allergic to penicillin; most strains in the previously mentioned study showed only intermediate susceptibility to erythromycin (102). Ciprofloxacin and the newer quinolone gatifloxacin had good in vitro activities against 40 Turkish isolates, but for another new quinolone,

levofloxacin, it was observed that MICs were high for 10 strains (43). Other in vitro studies have shown novel fluoroquinolones and a ketolide to be of potential therapeutic value (37, 48). Standards for antimicrobial susceptibility testing of *B. anthracis* have been recently adopted (28), and a DNA microarray for detecting antimicrobial resistance determinants in *B. anthracis* and *B. cereus* has been developed (7).

Postexposure prophylaxis (PEP) is needed for the prevention of inhalation anthrax following exposure to aerosols containing *B. anthracis* spores; the recommended regimen is 60 days of antimicrobial therapy and three doses of AVA (17), and recommended antimicrobial agents include ciprofloxacin, doxycycline, and levofloxacin. PEP was, for example, recommended for four persons who had been in the unventilated work space during procedures that generated aerosols from untreated animal hides used for making drums. Amoxicillin is recommended as an option in cases where the *B. anthracis* strain has been demonstrated to be susceptible to penicillins, and when other antimicrobial agents are not considered safe, as in the treatment of children and pregnant or lactating women (20, 21). The use of penicillins for PEP or for treatment of inhalation anthrax following the use of *B. anthracis* as a bioweapon gives cause for concern, owing to the presence of β -lactamases in *B. anthracis* isolates and the poor penetration of β -lactams into macrophages, the site of spore germination (9). Combination intravenous antimicrobial therapy with two or more antimicrobial agents, begun early, such as with ciprofloxacin and one or more other antimicrobial agents to which the organism is sensitive, appeared to improve survival rates during treatment of cases in the 2001 event in the United States (71). Following that event, the recommendation for initial treatment of inhalation anthrax is intravenous ciprofloxacin or doxycycline along with one or more agents to which the organism is normally susceptible (19); ciprofloxacin is favored over doxycycline as the primary antimicrobial agent due to its bactericidal action and central nervous system penetration in the event of meningeal involvement (129). A mouse aerosol challenge model has been developed for determining antimicrobial agent efficacy in the treatment of inhalation anthrax (62). In a case of inhalation anthrax that was naturally acquired when processing untreated animal hides for drum construction, the patient's therapy included adjunctive use of human anthrax immunoglobulin (147); however, the use of anthrax immunoglobulin in the treatment of a subsequent inhalation anthrax case in 2008 did not prevent a fatal outcome (3).

Bacillus cereus

There have been rather few studies of the antimicrobial susceptibility of *Bacillus cereus*, and most information has to be gleaned from reports of individual cases or outbreaks. *Bacillus cereus* and *B. thuringiensis* produce a broad-spectrum β -lactamase and are thus resistant to aminopenicillins and cephalosporins; they are also resistant to trimethoprim. An in vitro study of 54 isolates from blood cultures by disk diffusion assay found that all strains were susceptible to imipenem and vancomycin and that most were sensitive to chloramphenicol, ciprofloxacin, erythromycin, and gentamicin (but a small number of strains showed moderate or intermediate susceptibility), while 22 and 37% of strains showed only moderate or intermediate susceptibilities to clindamycin and tetracycline, respectively (148). Although strains are almost always susceptible to clindamycin, eryth-

romycin, chloramphenicol, vancomycin, and the aminoglycosides, and are usually sensitive to tetracycline and sulfonamides, there have been several reports of treatment failures with some of these drugs: a fulminant meningitis which did not respond to chloramphenicol (96); a fulminant infection in a neonate which was refractory to treatment that included vancomycin, gentamicin, imipenem, clindamycin, and ciprofloxacin (139); failure of vancomycin to eliminate the organism from CSF in association with a fluid shunt infection (11); vancomycin resistance in strains from respiratory specimens from pediatric intensive care patients (73); and persistent bacteremias with strains showing resistance to vancomycin in two hemodialysis patients (A. von Gottberg and W. van Nierop, personal communication). Oral ciprofloxacin has been used successfully in the treatment of *B. cereus* wound infections, bacteremia, and pulmonary infection, and in vitro activity has been shown for daptomycin (27). Clindamycin with gentamicin, given early, appears to be the best treatment for ophthalmic infections caused by *B. cereus*, and experiments with rabbits suggest that intravitreal corticosteroids and antimicrobials may be effective in such cases. CLSI document M45-A (29) tabulates antimicrobial breakpoints for aerobic, endospore-forming species.

Other Species

Information is sparse on treatment of infections with other species. Gentamicin was effective in treating a case of *B. licheniformis* ophthalmitis, vancomycin was successful in cases of *B. licheniformis* peritonitis in a CAPD patient (107) and a pacemaker wire infection, meropenem succeeded in a case of brain abscess, and cephalozin was effective against *B. licheniformis* prosthetic aortic valve endocarditis. Resistance to macrolides appears to occur naturally in *B. licheniformis*. *B. subtilis* endocarditis in a drug abuser was successfully treated with a cephalosporin, and gentamicin was successful against *B. subtilis* septicemia. A *B. pumilus* central venous catheter infection treated with several drugs, including flucloxacillin, clindamycin, and vancomycin, failed to clear, and the catheter had to be replaced with a new one (10). Two cases of cutaneous infections with *B. pumilus* initially diagnosed as anthrax were treated successfully with amoxicillin-clavulanate, while a third such case was treated with ciprofloxacin (134). Daptomycin and ciprofloxacin show in vitro activity against strains of *B. pumilus*, *B. subtilis*, and some other species (27). Penicillin, or its derivatives, or cephalosporins probably have long been the first choices for treatment of infections attributed to other *Bacillus* species. However, in a study by Weber et al. (148), over 95% of isolates of *B. megaterium*, *B. pumilus*, *B. subtilis*, *B. circulans*, *B. amyloliquefaciens*, and *B. licheniformis*, along with strains of *B. (now Paenibacillus) polymyxa* and three unidentified strains from blood cultures, were susceptible to imipenem, ciprofloxacin, and vancomycin, and only between 75 and 90% were susceptible to penicillins, cephalosporins, and chloramphenicol. Isolates of "*B. polymyxa*" and *B. circulans* were more likely to be resistant to the penicillins and cephalosporins than strains of the other species—it is probable that some or all of the strains identified as *B. circulans* might now be accommodated in *Paenibacillus*, along with "*B. polymyxa*." An infection of a human bite wound with an organism identified as *B. circulans* did not respond to treatment with amoxicillin and flucloxacillin but was resolved with clindamycin, while peritonitis in a CAPD patient was resolved with vancomycin. However, a strain identified

as *B. circulans* and showing vancomycin resistance has been isolated from an Italian clinical specimen (88). Vancomycin resistance has been reported for *P. popilliae*, a biopesticide, and isolates of this species have been shown to carry genes resembling those responsible for high-level vancomycin resistance in enterococci (108). Of two South African vancomycin-resistant clinical isolates, one was identified as *Paenibacillus thiaminolyticus* and the other was unidentified but considered to be related to *Bacillus lentus*. The latter was isolated from a case of neonatal sepsis and has been shown to have inducible resistance to vancomycin and teicoplanin; this is in contrast to the *B. circulans* and *P. thiaminolyticus* isolates mentioned above, in which expression of resistance was found to be constitutive (von Gottberg and van Nierop, personal communication).

VACCINATION

AVA, the current human vaccine in the United States, is a cell-free filtrate (formalin treated), in an aluminum hydroxide-adsorbed gel, from a noncapsulated, nonproteolytic derivative of strain V770-NP1_R grown under microaerobic conditions. The FDA has approved a new route and schedule for preexposure immunization with AVA, which calls for a series of five intramuscular injections administered at 0 and 4 weeks and at 6, 12, and 18 months (95). To maintain immunity, an annual booster injection is recommended. Anthrax vaccine precipitated, the current human vaccine in the United Kingdom, is an alum-precipitated cell-free filtrate of Sterne strain (34F2) cultured, under static batch conditions, with activated charcoal to increase PA production. Both anthrax vaccine precipitated (150) and AVA contain PA as well as trace amounts of LF, EF, and cell wall proteins. In October 2008 the ACIP voted to accept provisional recommendations for the pre-event use of anthrax vaccine among persons considered to be at risk for exposure to aerosolized *B. anthracis* spores; these provisional recommendations included new language to address emergency responders, in addition to the previously approved recommended occupational and laboratory populations. The ACIP recommends routine preexposure vaccination with AVA for persons engaged in work involving (i) production of high concentrations or pure cultures of *B. anthracis* spores, (ii) activities with a high potential for production of aerosolized spores, (iii) handling of environmental samples associated with anthrax investigations (especially powders) and performance of confirmatory testing for *B. anthracis* in the U.S. LRN for bioterrorism level B laboratories or above, (iv) making repeated entries into known *B. anthracis* spore-contaminated areas or settings in which repeated exposure to aerosolized *B. anthracis* spores might occur, and (v) engaging in environmental investigations or remediation efforts of spore-contaminated areas or other settings with aerosol exposure. Immunization is not routinely recommended for emergency and other responders but may be offered on a voluntary basis as part of a comprehensive occupational health and safety program. Laboratory workers using standard biosafety level 2 practices in the routine processing of clinical or environmental specimens in general diagnostic laboratories are not at increased risk for exposure to *B. anthracis* spores (22, 25). The development of anthrax vaccines, including second- and third-generation products, and that of several human monoclonal and polyclonal antibody products currently in their early development and testing phases are the subjects of recent reviews (120, 138).

EVALUATION, INTERPRETATION, AND REPORTING OF RESULTS

While, of course, the isolation of *B. anthracis* is always significant and requires urgent reporting, the majority of other aerobic endospore-forming species are environmental organisms, and so they are frequent laboratory contaminants. Therefore, isolation from a single clinical specimen is generally not a sufficient basis for incriminating one of these organisms as the etiological agent; however, any such organism should be considered of potential clinical significance if it is isolated in pure culture or at least apparently dominating the microbiota, or if it is isolated in large numbers or isolated more than once. Opportunistic infections with *Bacillus* species other than *B. anthracis* have been reported since the late 19th century, and in the last 30 years the clinical importance of aerobic endospore formers (most, but not all, of them *Bacillus* species) has become widely accepted. It is most important to assess any clinical isolation of such an organism in the light of any other species cultured and the clinical context and to be wary of dismissing it as a mere contaminant. Moderate or heavy growth of aerobic endospore formers from wounds is usually significant, and *B. cereus* infections of the eye are serious emergencies that should always be reported to the physician immediately.

Low-level contamination of foodstuffs by aerobic endospore formers is commonplace, as is asymptomatic transient fecal carriage. Therefore, in foodborne illness investigations, qualitative isolation tests are insufficient. The ideal criteria for establishing that an aerobic endospore former is the etiological agent are (i) isolation of significant numbers ($>10^5$ CFU/g) of the organism from the epidemiologically incriminated food (and, in the case of suspected *B. cereus* food poisoning, detection of emetic toxin and/or enterotoxin) and (ii) recovery of the same strain (biovar, plasmid type, etc.) in significant numbers from acute-phase specimens (feces or vomitus) from the patients but not from healthy controls.

REFERENCES

1. Abusin, S., A. Bhimaraj, and S. Khadra. 2008. *Bacillus cereus* endocarditis in a permanent pacemaker: a case report. *Cases J.* 1:95.
2. Ahmed, I., A. Yokota, A. Yamazoe, and T. Fujiwara. 2007. Proposal of *Lysinibacillus boronitolerans* gen. nov. sp. nov., and transfer of *Bacillus fusiformis* to *Lysinibacillus fusiformis* comb. nov. and *Bacillus sphaericus* to *Lysinibacillus sphaericus* comb. nov. *Int. J. Syst. Evol. Microbiol.* 57:1117–1125.
3. Anaraki, S., S. Addiman, G. Nixon, D. Krahé, R. Ghosh, T. Brooks, G. Lloyd, R. Spencer, A. Walsh, B. McCloskey, and N. Lightfoot. 2008. Investigations and control measures following a case of inhalation anthrax in East London in a drum maker and drummer, October 2008. *Eurosurveillance* 13:1–3.
4. Andersson, M. A., R. Mikkola, J. Helin, M. C. Andersson, and M. Salkinoja-Salonen. 1998. A novel sensitive bioassay for detection of *Bacillus cereus* emetic toxin and related depi-peptide ionophores. *Appl. Environ. Microbiol.* 64:1338–1343.
5. Andreotti, P. E., G. V. Ludwig, A. H. Peruski, J. J. Tuite, S. S. Morse, and L. F. Peruski. 2003. Immunoassay of infectious agents. *BioTechniques* 35:850–859.
6. Ankolekar, C., T. Rahmati, and R. G. Labbe. 2009. Detection of toxigenic *Bacillus cereus* and *Bacillus thuringiensis* spores in U.S. rice. *Int. J. Food Microbiol.* 128:460–466.
7. Antwerpen, M. H., M. Schellhase, E. Ehrentreich-Förster, F. Bier, W. Witte, and U. Nübel. 2007. DNA microarray for detection of antimicrobial resistance determinants in *Bacillus anthracis* and closely related *Bacillus cereus*. *Mol. Cell. Probes* 21:152–160.
8. Bell, C. A., J. R. Uhl, T. L. Hadfield, J. C. David, R. F. Meyer, T. F. Smith, and F. R. Cockerill. 2002. Detection of

- Bacillus anthracis* DNA by LightCycler PCR. *J. Clin. Microbiol.* 40:2897–2902.
9. Bell, D. M., S. Blank, P. E. Kozarsky, and D. S. Stephens. 2002. Clinical issues in the prophylaxis, diagnosis, and treatment of anthrax. *Emerg. Infect. Dis.* 8:222–225.
 10. Bentur, H. N., A. M. Dalzell, and F. A. I. Riordan. 2007. Central venous catheter infection with *Bacillus pumilus* in an immunocompetent child: a case report. *Ann. Clin. Microbiol. Antimicrobials* 6:12.
 11. Berne, R., F. Heinen, K. Pelz, V. van Velthoven, M. Sauer, and R. Korinthenberg. 1997. Ventricular shunt infection and meningitis due to *Bacillus cereus*. *Neuropediatrics* 28:333–334.
 12. Berry, N. I., Hassan, S. Majumdar, A. Vardhan, A. McEwen, and R. Gokal. 2004. *Bacillus circulans* peritonitis in a patient treated with CAPD. *Peritoneal Dial. Int.* 24:488–489.
 13. Bishop, A. H. 2002. *Bacillus thuringiensis* insecticides, p. 160–175. In R. W. Berkeley, M. Heyndrickx, N. A. Logan, and P. De Vos (ed.), *Applications and Systematics of Bacillus and Relatives*. Blackwell Science, Oxford, United Kingdom.
 14. Bosshard, P. P., R. Zbinden, and M. Altwegg. 2002. *Paenibacillus turicensis* sp. nov., a novel bacterium harbouring heterogeneities between 16S rRNA genes. *Int. J. Syst. Evol. Microbiol.* 52:2241–2249.
 15. Boyer, A. E., C. P. Quinn, A. R. Woolfitt, J. L. Pirkle, L. G. McWilliams, K. L. Stamey, D. A. Bagarozzi, J. C. Hart, Jr., and J. R. Barr. 2007. Detection and quantification of anthrax lethal factor in serum by mass spectrometry. *Anal. Chem.* 79:8463–8470.
 16. Brachman, P., and A. Kaufmann. 1998. Anthrax, p. 95–107. In A. Evans and P. Brachman (ed.), *Bacterial Infections of Humans*. Plenum Medical Book Company, New York, NY.
 17. Callegan, M. C., S. T. Kane, D. C. Cochran, B. Novosad, M. S. Gilmore, M. Gominet, and D. Lereclus. 2005. *Bacillus* endophthalmitis: roles of bacterial toxins and motility during infection. *Investig. Ophthalmol. Vis. Sci.* 46:3233–3238.
 18. Cardazzo, B., E. Negrisola, L. Carraro, L. Alberghini, T. Patarnello, and V. Giaccone. 2008. Multiple-locus sequence typing and analysis of toxin genes in *Bacillus cereus* food-borne isolates. *Appl. Environ. Microbiol.* 74:850–860.
 19. Centers for Disease Control and Prevention. 2001. Update: investigation of bioterrorism-related anthrax and interim guidelines for exposure management and antimicrobial therapy. *MMWR Morb. Mortal. Wkly. Rep.* 50:909.
 20. Centers for Disease Control and Prevention. 2001. Updated recommendations for antimicrobial prophylaxis among asymptomatic pregnant women after exposure to *Bacillus anthracis*. *MMWR Morb. Mortal. Wkly. Rep.* 50:960.
 21. Centers for Disease Control and Prevention. 2001. Interim recommendations for antimicrobial prophylaxis for children and breastfeeding mothers and treatment of children with anthrax. *MMWR Morb. Mortal. Wkly. Rep.* 50:1014–1016.
 22. Centers for Disease Control and Prevention. 2002. Notice to readers: use of anthrax vaccine in response to terrorism: supplemental recommendations of the Advisory Committee on Immunization Practices. *MMWR Morb. Mortal. Wkly. Rep.* 51:1024–1026.
 23. Centers for Disease Control and Prevention. 2008. Cutaneous anthrax associated with drum making using goat hides from West Africa—Connecticut, 2007. *MMWR Morb. Mortal. Wkly. Rep.* 57:628–631.
 24. Centers for Disease Control and Prevention. 2009. Surveillance for foodborne disease outbreaks—United States, 2006. *MMWR Morb. Mortal. Wkly. Rep.* 58:609–615.
 25. Centers for Disease Control and Prevention. 2009. Advisory Committee on Immunization Practices (ACIP) Provisional Recommendations for Use of Anthrax Vaccine Adsorbed. Centers for Disease Control and Prevention, Atlanta, GA. <http://www.cdc.gov/vaccines/recs/provisional/downloads/anthrax-vax-oct2009-508.pdf>.
 26. Centers for Disease Control and Prevention and National Institutes of Health. 2007. *Biosafety in Microbiological and Biomedical Laboratories*, 5th ed. U.S. Government Printing Office, Washington, DC.
 27. Citron, D. M., and M. D. Appleman. 2006. In vitro activities of daptomycin, ciprofloxacin, and other antimicrobial agents against the cells and spores of clinical isolates of *Bacillus* species. *J. Clin. Microbiol.* 44:3814–3818.
 28. Clinical and Laboratory Standards Institute. 2005. *Performance Standards for Antimicrobial Susceptibility Testing: 15th Informational Supplement*. CLSI document M100-S15. Clinical and Laboratory Standards Institute, Wayne, PA.
 29. Clinical and Laboratory Standards Institute. 2006. *Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria*. CLSI/NCCLS Approved Guideline M45-A. Clinical and Laboratory Standards Institute, Wayne, PA.
 30. Coker, P. R., K. L. Smith, and M. E. Hugh-Jones. 2002. Antimicrobial susceptibilities of diverse *Bacillus anthracis* isolates. *Antimicrob. Agents Chemother.* 46:3843–3845.
 31. Cote, C. K., T. L. DiMezzo, D. J. Banks, B. France, K. A. Bradley, and S. L. Welkos. 2008. Early interactions between fully virulent *Bacillus anthracis* and macrophages that influence the balance between spore clearance and development of a lethal infection. *Microb. Infect. Inst. Pasteur* 10:613–619.
 32. Darbar, A., I. A. Harris, and I. B. Gosbell. 2005. Necrotizing infection due to *Bacillus cereus* mimicking gas gangrene following penetrating trauma. *J. Orthop. Trauma* 19:353–355.
 33. De, B. K., S. L. Bragg, G. N. Sanden, K. E. Wilson, L. A. Diem, C. K. Marston, A. R. Hoffmaster, G. A. Barnett, R. S. Weyant, T. G. Abshire, J. W. Ezzell, and T. Popovic. 2002. Two-component direct fluorescent-antibody assay for rapid identification of *Bacillus anthracis*. *Emerg. Infect. Dis.* 8:1060–1065.
 34. Didelot, X., M. Barker, D. Falush, and F. G. Priest. 2009. Evolution of pathogenicity in the *Bacillus cereus* group. *Syst. Appl. Microbiol.* 32:81–90.
 35. Dohmae, S., T. Okubo, W. Higuchi, T. Takano, H. Isobe, T. Baranovich, S. Kobayashi, M. Uchiyama, Y. Tanabe, M. Itoh, and T. Yamamoto. 2008. *Bacillus cereus* nosocomial infection from reused towels in Japan. *J. Hosp. Infect.* 69:361–367.
 36. Doran, M., D. S. Raicu, J. D. Furst, R. Settimi, M. Schipma, and D. P. Chandler. 2007. Oligonucleotide microarray identification of *Bacillus anthracis* strains using support vector machines. *Bioinformatics* 23:487–492.
 37. Drago, L., E., De Vecchi, A. Lombardi, L. Incola, M. Valli, and M. R. Gismondo. 2002. Bactericidal activity of levofloxacin, gatifloxacin, penicillin, meropenem and rifampin against *Bacillus anthracis* clinical isolates. *J. Antimicrob. Chemother.* 50:1059–1063.
 38. Dubouix, A., E. Bonnet, M. Alvarez, H. Bensafi, M. Archambaud, B. Chaminade, G. Chabanon, and N. Marty. 2005. *Bacillus cereus* infections in traumatology-orthopaedics department: retrospective investigation and improvement of healthcare practices. *J. Infect.* 50:22–30.
 39. Easterday, W. R., M. N. Van Ert, S. Zanecki, and P. Keim. 2005. Specific detection of *Bacillus anthracis* using a TaqMan mismatch amplification mutation assay. *BioTechniques* 38:731–735.
 40. Editorial team. 2006. Probable human anthrax death in Scotland. *Eurosurveillance* 11:pii=3025.
 41. Ehling-Schulz, M., M. Fricker, H. Grallert, P. Rieck, M. Wagner, and S. Scherer. 2006. Cereulide synthetase gene cluster from emetic *Bacillus cereus*: structure and location on a mega virulence plasmid related to *Bacillus anthracis* toxin plasmid pXO1. *BMC Microbiol.* 6:20.
 42. El Saleeby, C. M., S. C. Howard, R. T. Hayden, and J. A. McCullers. 2004. Association between tea ingestion and invasive *Bacillus cereus* infection among children with cancer. *Clin. Infect. Dis.* 39:1536–1539.
 43. Esel, D., M. Doganay, and B. Sumerkan. 2003. Antimicrobial susceptibilities of 40 isolates of *Bacillus anthracis* isolated in Turkey. *Int. J. Antimicrob. Agents* 22:70–72.
 44. Evreux, F., B. Delaporte, N. Leret, C. Buffet-Janvresse, and A. Morel. 2007. A case of fatal neonatal *Bacillus cereus* meningitis. *Arch. Pediatr.* 14:365–368. (In French.)

45. Ezzell, J. W., Jr., T. G. Abshire, S. F. Little, B. C. Lidgerding, and C. Brown. 1990. Identification of *Bacillus anthracis* by using monoclonal antibody to cell wall galactose-*N*-acetylglucosamine polysaccharide. *J. Clin. Microbiol.* 28:223–231.
46. Finlay, W. J. J., N. A. Logan, and A. D. Sutherland. 1999. Semiautomated metabolic staining assay for *Bacillus cereus* emetic toxin. *Appl. Environ. Microbiol.* 65:1811–1812.
47. Frankard, J., R. Li, F. Taccone, M. J. Struelens, F. Jacobs, and A. Kentos. 2004. *Bacillus cereus* pneumonia in a patient with acute lymphoblastic leukemia. *Eur. J. Clin. Microbiol. Infect. Dis.* 23:725–728.
48. Frean, J., K. P. Klugman, L. Arntzen, and S. Bukofzer. 2003. Susceptibility of *Bacillus anthracis* to eleven antimicrobial agents including novel fluoroquinolones and a ketolide. *J. Antimicrob. Chemother.* 52:297–299.
49. Fricker, M., U. Messelhäuser, U. Busch, S. Scherer, and M. Ehling-Schulz. 2007. Diagnostic real-time PCR assays for the detection of emetic *Bacillus cereus* strains in foods and recent food-borne outbreaks. *Appl. Environ. Microbiol.* 73:1892–1898.
50. From, C., V. Hormazabal, and P. E. Granum. 2007. Food poisoning associated with pumilacidin-producing *Bacillus pumilus* in rice. *Int. J. Food Microbiol.* 115:319–324.
51. Galanos, J., S. Perera, H. Smith, D. O'Neal, H. Sheorey, and M. J. Waters. 2003. Bacteremia due to three *Bacillus* species in a case of Munchausen's syndrome. *J. Clin. Microbiol.* 41:2247–2248.
52. Gerberding, J. L., J. M. Hughes, and J. P. Koplan. 2002. Bioterrorism preparedness and response: clinicians and public health agencies as essential partners. *JAMA* 287:898–900.
53. Ghelardi, E., F. Celandroni, S. Salvetti, C. Barsotti, A. Baggiani, and S. Senesi. 2002. Identification and characterization of toxigenic *Bacillus cereus* isolates responsible for two food-poisoning outbreaks. *FEMS Microbiol. Lett.* 208:129–134.
54. Ghelardi, E., F. Celandroni, S. Salvetti, E. Fiscarelli, and S. Senesi. 2007. *Bacillus thuringiensis* pulmonary infection: critical role for bacterial membrane-damaging toxins and host neutrophils. *Microbes Infect.* 9:591–598.
55. Gill, S. R., D. E. Fouts, G. L. Archer, E. F. Mongodin, R. T. Deboy, J. Ravel, I. T. Paulsen, J. F. Kolonay, L. Brinkac, M. Beanan, R. J. Dodson, S. C. Daugherty, R. Madupu, S. V. Angiuoli, A. S. Durkin, D. H. Haft, J. Vamathevan, H. Khouri, T. Utterback, C. Lee, G. Dimitrov, L. Jiang, H. Qin, J. Weidman, K. Tran, K. Kang, I. R. Hance, K. E. Nelson, and C. M. Fraser. 2005. Insights on evolution of virulence and resistance from the complete genome analysis of an early methicillin-resistant *Staphylococcus aureus* strain and a biofilm-producing methicillin-resistant *Staphylococcus epidermidis* strain. *J. Bacteriol.* 187:2426–2438.
56. Glazunova, O. O., D. Raoult, and V. Roux. 2006. *Bacillus massiliensis* sp. nov., isolated from cerebrospinal fluid. *Int. J. Syst. Evol. Microbiol.* 56:1485–1488.
57. Gürkan, S., F. Akata, F. Kuloğlu, S. Erdoğan, and M. Tuğrul. 2005. Meningitis due to *Bacillus anthracis*. *Yonsei Med. J.* 46:159–160.
58. Haase, R., H. Sauer, U. Dagwadordsch, J. Foell, and U. Lieser. 2005. Successful treatment of *Bacillus cereus* meningitis following allogeneic stem cell transplantation. *Pediatr. Transplant.* 9:338–341.
59. Häggblom, M. M., C. Apetroaie, M. A. Andersson, and M. S. Salkinoja-Salonen. 2002. Quantitative analysis of cereulide, the emetic toxin of *Bacillus cereus*, produced under various conditions. *Appl. Environ. Microbiol.* 68:2479–2483.
60. Hahn, B. L., S. Sharma, and P. G. Sohnle. 2005. Analysis of epidermal entry in experimental cutaneous *Bacillus anthracis* infections in mice. *J. Lab. Clin. Med.* 146:95–102.
61. Hannah, W., and P. T. Ender. 1999. Persistent *Bacillus licheniformis* bacteraemia associated with an intentional injection of organic drain cleaner. *Clin. Infect. Dis.* 29:659–661.
62. Heine, H. S., J. Bassett, L. Miller, J. M. Hartings, B. E. Ivins, M. L. Pitt, D. Fritz, S. L. Norris, and W. R. Byrne. 2007. Determination of antibiotic efficacy against *Bacillus anthracis* in a mouse aerosol challenge model. *Antimicrob. Agents Chemother.* 51:1373–1379.
63. Helgason, E., D. A. Caugant, I. Olsen, and A.-B. Kolstø. 2000. Genetic structure of population of *Bacillus cereus* and *B. thuringiensis* isolates associated with periodontitis and other human infections. *J. Clin. Microbiol.* 38:1615–1622.
64. Helgason, E., N. J. Tourasse, R. Meisal, D. A. Caugant, and A. B. Kolstø. 2004. Multilocus sequence typing scheme for bacteria of the *Bacillus cereus* group. *Appl. Environ. Microbiol.* 70:191–201.
65. Hernaiz, C., A. Picardo, J. I. Alos, and J. L. Gomez-Garcés. 2003. Nosocomial bacteremia and catheter infection by *Bacillus cereus* in an immunocompetent patient. *Clin. Microbiol. Infect.* 9:973–975.
66. Hoffmaster, A. R., R. F. Meyer, M. D. Bowen, C. K. Marston, R. S. Weyant, K. Thurman, S. L. Messenger, E. E. Minor, J. M. Winchell, M. V. Rassmussen, B. R. Newton, J. T. Parker, W. E. Morrill, N. McKinney, G. A. Barnett, J. J. Sejvar, J. A. Jernigan, B. A. Perkins, and T. Popovic. 2002. Evaluation and validation of a real-time polymerase chain reaction assay for rapid identification of *Bacillus anthracis*. *Emerg. Infect. Dis.* 8:1178–1182.
67. Hoffmaster, A. R., K. K. Hill, J. E. Gee, C. K. Marston, B. K. De, T. Popovic, D. Sue, P. P. Wilkins, S. B. Avashia, R. Drumgoole, C. H. Helma, L. O. Ticknor, R. T. Okinaka, and P. J. Jackson. 2006. Characterization of *Bacillus cereus* isolates associated with fatal pneumonias: isolates are closely related to *Bacillus anthracis* and harbor *B. anthracis* virulence genes. *J. Clin. Microbiol.* 44:3352–3360.
68. Hoffmaster, A. R., R. T. Novak, C. K. Marston, J. E. Gee, L. Helsel, J. M. Pruckler, and P. P. Wilkins. 2008. Genetic diversity of clinical isolates of *Bacillus cereus* using multilocus sequence typing. *BMC Microbiol.* 8:191.
69. Hurtle, W., E. Bode, R. S. Kaplan, J. Garrison, B. Kearney, D. Shoemaker, E. Henchal, and D. Norwood. 2003. Use of denaturing high-performance liquid chromatography to identify *Bacillus anthracis* by analysis of the 16S-23S rRNA interspacer region and *gyrA* gene. *J. Clin. Microbiol.* 41:4758–4766.
70. Hurtle, W., E. Bode, D. A. Kulesh, R. S. Kaplan, J. Garrison, D. Bridge, M. House, M. S. Frye, B. Loveless, and D. Norwood. 2004. Detection of the *Bacillus anthracis gyrA* gene by using a minor groove binder probe. *J. Clin. Microbiol.* 42:179–185.
71. Jernigan, J. A., D. S. Stephens, D. A. Ashford, C. Omenaca, M. S. Topiel, M. Galbraith, M. Tapper, T. L. Fisk, S. Zaki, T. Popovic, R. F. Meyer, C. P. Quinn, S. A. Harper, S. K. Fridkin, J. J. Sejvar, C. W. Shepard, M. McConnell, J. Guarner, W.-J. Shieh, J. M. Malecki, J. L. Gerberding, J. M. Hughes, and B. A. Perkins. 2002. Anthrax bioterrorism investigation. Bioterrorism-related inhalational anthrax: the first 10 cases reported in the United States. *Emerg. Infect. Dis.* 7:933–944.
72. Juergensmeyer, M. A., B. A. Gingras, L. Restaino, and E. W. Frampton. 2006. A selective chromogenic agar that distinguishes *Bacillus anthracis* from *Bacillus cereus* and *Bacillus thuringiensis*. *J. Food. Prot.* 69:2002–2006.
73. Kalpoe, J. S., K. Hogenbirk, N. M. van Maarseveen, B. J. Gesink-Van der Veer, M. E. M. Kraakman, J. J. Maarleveld, T. J. K. van der Reyden, L. Dijkshoorn, and A. T. Bernards. 2008. Dissemination of *Bacillus cereus* in a paediatric intensive care unit traced to insufficient disinfection of reusable ventilator air-flow sensors. *J. Hosp. Infect.* 68:341–347.
74. Kämpfer, P., R. Rosselló-Mora, E. Falsen, H.-J. Busse, and B. J. Tindall. 2006. *Cohnella thermotolerans* gen. nov., sp. nov., and classification of 'Paenibacillus hongkongensis' as *Cohnella hongkongensis* sp. nov. *Int. J. Syst. Evol. Microbiol.* 56:781–786.
75. Kawamura-Sato, K., Y. Hirama, N. Agata, H. Ito, K. Torii, A. Takeno, T. Hasegawa, Y. Shimomura, and M. Ohta. 2005. Quantitative analysis of cereulide, an emetic toxin of *Bacillus cereus*, by using rat liver mitochondria. *Microbiol. Immunol.* 49:25–30.
76. Keim, P., L. B. Price, A. M. Klevytska, K. L. Smith, J. M. Schupp, R. Okinaka, P. J. Jackson, and M. E. Hugh-Jones. 2000. Multiple-locus variable-number tandem repeat analysis

- reveals genetic relationships within *Bacillus anthracis*. *J. Bacteriol.* 182:2928–2936.
77. Kenefic, L. J., J. Beaudry, C. Trim, R. Daly, R. Parmar, S. Zanecki, L. Huynh, M. N. Van Ert, D. M. Wagner, T. Graham, and P. Keim. 2008. High resolution genotyping of *Bacillus anthracis* outbreak strains using four highly mutable single nucleotide repeat markers. *Lett. Appl. Microbiol.* 46:600–603.
 78. Kenefic, L. J., J. Beaudry, C. Trim, L. Huynh, S. Zanecki, M. Matthews, J. Schupp, M. Van Ert, and P. Keim. 2008. A high resolution four-locus multiplex single nucleotide repeat (SNR) genotyping system in *Bacillus anthracis*. *J. Microbiol. Methods* 73:269–272.
 79. Klee, S. R., M. Ozel, B. Appel, C. Boesch, H. Ellerbrok, D. Jacob, G. Holland, F. H. Leendertz, G. Pauli, R. Grunow, and H. Nattermann. 2006. Characterization of *Bacillus anthracis*-like bacteria isolated from wild great apes from Côte d'Ivoire and Cameroon. *J. Bacteriol.* 188:5333–5344.
 80. Knisely, R. F. 1966. Selective medium for *Bacillus anthracis*. *J. Bacteriol.* 92:784–786.
 81. Ko, K. S., J. W. Kim, J. M. Kim, W. Kim, S. I. Chung, I. J. Kim, and Y. H. Kook. 2004. Population structure of the *Bacillus cereus* group as determined by sequence analysis of six housekeeping genes and the *plcR* gene. *Infect. Immun.* 72:5253–5261.
 82. Ko, K. S., W. S. Oh, M. Y. Lee, J. H. Lee, H. Lee, K. R. Peck, N. Y. Lee, and J.-H. Song. 2006. *Bacillus infantis* sp. nov. and *Bacillus idriensis* sp. nov., isolated from a patient with neonatal sepsis. *Int. J. Syst. Evol. Microbiol.* 56:2541–2544.
 83. Ko, K. S., Y.-S. Kim, M. Y. Lee, S. Y. Shin, D. S. Jung, K. R. Peck, and J.-H. Song. 2008. *Paenibacillus konsidensis* sp. nov., isolated from a patient. *Int. J. Syst. Evol. Microbiol.* 58:2164–2168.
 84. Ko, S.-Y., H. J. Chung, H.-S. Sung, and M.-N. Kim. 2007. Emergence of beta-lactam-dependent *Bacillus cereus* associated with prolonged treatment with cefepime in a neutropenic patient. *Korean J. Lab. Med.* 27:216–220. (In Korean.)
 85. Kolstø, A.-B., N. J. Tourasse, and O. A. Økstad. 2009. What sets *Bacillus anthracis* apart from other *Bacillus* species? *Annu. Rev. Microbiol.* 63:451–476.
 86. Lalitha, M. K., V. Anandi, N. Walter, J. O. Devadatta, and B. M. Pulimood. 1988. Primary anthrax presenting as an injection "abscess." *Indian J. Pathol. Microbiol.* 31:254–256.
 87. Le Fleche, P., Y. Hauck, L. Onteniente, A. Prieur, F. Denoel, V. Ramière, P. Sylvestre, G. Benson, F. Ramière, and G. Vergnaud. 2001. A tandem repeats database for bacterial genomes: application to the genotyping of *Yersinia pestis* and *Bacillus anthracis*. *BMC Microbiol.* 1:2.
 88. Ligozzi, M., G. L. Cascio, and R. Fontana. 1998. *vanA* gene cluster in a vancomycin-resistant clinical isolate of *Bacillus circulans*. *Antimicrob. Agents Chemother.* 42:2055–2059.
 89. Lista, F., G. Faggioli, S. Valjevac, A. Ciammarucini, J. Vaissaire, C. le Doujet, G. Gorge, R. De Santis, A. Carattoli, A. Ciervo, A. Fasanella, F. Orsini, R. D'Amelio, C. Pourcel, A. Cassone, and G. Vergnaud. 2006. Genotyping of *Bacillus anthracis* strains based on automated capillary 25-loci multiple locus variable-number tandem repeats analysis. *BMC Microbiol.* 6:33.
 90. Logan, N. A., and P. De Vos. 2009. *Bacillus*, p. 21–128. In P. De Vos, G. M. Garrity, D. Jones, N. R. Krieg, W. Ludwig, F. R. Rainey, K.-H. Schleifer, and W. B. Whitman (ed.), *Bergey's Manual of Systematic Bacteriology*, 2nd ed., vol. 3. Springer, New York, NY.
 91. Logan, N. A., and P. De Vos. 2009. *Brevibacillus*, p. 305–316. In P. De Vos, G. M. Garrity, D. Jones, N. R. Krieg, W. Ludwig, F. R. Rainey, K.-H. Schleifer, and W. B. Whitman (ed.), *Bergey's Manual of Systematic Bacteriology*, 2nd ed., vol. 3. Springer, New York, NY.
 92. Logan, N. A., P. De Vos, and A. E. Dinsdale. 2009. *Geobacillus*, p. 144–160. In P. De Vos, G. M. Garrity, D. Jones, N. R. Krieg, W. Ludwig, F. R. Rainey, K.-H. Schleifer, and W. B. Whitman (ed.), *Bergey's Manual of Systematic Bacteriology*, 2nd ed., vol. 3. Springer, New York, NY.
 93. Logan, N. A., G. Forsyth, L. Lebbe, J. Goris, M. Heyndrickx, A. Balcaen, A. Verhelst, E. Falsen, Å. Ljungh, H. B. Hansson, and P. De Vos. 2002. Polyphasic identification of *Bacillus* and *Brevibacillus* strains from clinical, dairy, and industrial specimens and proposal of *Brevibacillus invocatus* sp. nov. *Int. J. Syst. Evol. Microbiol.* 52:953–966.
 94. Mahler, H., A. Pasi, J. M. Kramer, P. Schulte, A. C. Scoging, W. Bär, and S. Krähenbühl. 1997. Fulminant liver failure in association with the emetic toxin of *Bacillus cereus*. *N. Engl. J. Med.* 336:1142–1148.
 95. Marano, N., B. D. Plikaytis, S. W. Martin, C. Rose, V. A. Semenova, S. K. Martin, A. E. Freeman, H. Li, M. J. Mulligan, S. D. Parker, J. Babcock, W. Keitel, H. El Sahly, G. A. Poland, R. M. Jacobson, H. L. Keyserling, S. D. Soroka, S. P. Fox, J. L. Stamper, M. M. McNeil, B. A. Perkins, N. Messonnier, and C. P. Quinn. 2008. Effects of a reduced dose schedule and intramuscular administration of anthrax vaccine adsorbed on immunogenicity and safety at 7 months: a randomized trial. *JAMA* 300:1532–1543.
 96. Marshman, L. A. G., C. Hardwidge, and P. M. W. Donaldson. 2000. *Bacillus cereus* meningitis complicating cerebrospinal fluid fistula repair and spinal drainage. *Br. J. Neurosurg.* 14:580–582.
 97. Marston, C. K., C. Beesley, L. Helsel, and A. R. Hoffmaster. 2008. Evaluation of two selective media for the isolation of *Bacillus anthracis*. *Lett. Appl. Microbiol.* 47:25–30.
 98. McIntyre, L., K. Bernard, D. Beniac, J. L. Isaac-Renton, and D. C. Naseby. 2008. Identification of *Bacillus cereus* group species associated with food poisoning outbreaks in British Columbia, Canada. *Appl. Environ. Microbiol.* 74:7451–7453.
 99. Mikkola, R., M. Kolari, M. A. Andersson, J. Helin, and M. S. Salkinoja-Salonen. 2000. Toxic lactonic lipopeptide from food poisoning isolates of *Bacillus licheniformis*. *Eur. J. Biochem.* 267:4068–4074.
 100. Mikkola, R., M. A. Andersson, P. Grigoriev, V. V. Teplova, N.-E. L. Saris, F. A. Rainey, and M. S. Salkinoja-Salonen. 2004. Toxic lactonic lipopeptide from food poisoning isolates of *Bacillus amyloliquefaciens* strains isolated from moisture-damaged buildings produced surfactin and a substance toxic to mammalian cells. *Arch. Microbiol.* 181:314–323.
 101. Miller, J. J., I. U. Scott, H. W. Flynn, W. E. Smiddy, T. G. Murray, A. Berrocal, and D. Miller. 2008. Endophthalmitis caused by *Bacillus* species. *Am. J. Ophthalmol.* 145:883–888.
 102. Mohammed, M. J., C. H. Marston, T. Popovic, R. S. Weyant, and F. C. Tenover. 2002. Antimicrobial susceptibility testing of *Bacillus anthracis*: comparison of results obtained by using the National Committee for Clinical Laboratory Standards broth microdilution reference and Etest agar gradient diffusion methods. *J. Clin. Microbiol.* 40:1902–1907.
 103. Morse, S. A., R. B. Kellogg, S. Perry, R. F. Meyer, D. Bray, D. Nicholson, and J. M. Miller. 2003. Detecting biothreat agents: the Laboratory Response Network. *ASM News* 69:433–437.
 104. Muller, J. D., C. R. Wilks, K. J. O'Riley, R. J. Condron, R. Bull, and A. Mateczun. 2004. Specificity of an immunochromatographic test for anthrax. *Aust. Vet. J.* 82:220–222.
 105. Ngamwongsatit, P., W. Buasri, P. Pianariyanon, C. Pulsrikarn, M. Ohba, A. Assavanig, and W. Panbangred. 2008. Broad distribution of enterotoxin genes (*hblCDA*, *nheABC*, *cytK*, and *entFM*) among *Bacillus thuringiensis* and *Bacillus cereus* as shown by novel primers. *Int. J. Food Microbiol.* 121:352–356.
 106. Ozkocaman, V., T. Ozcelik, R. Ali, F. Ozkalemkas, A. Ozkan, C. Ozakin, H. Akalin, A. Ursavas, F. Coskun, and B. Ener. 2006. *Bacillus* spp. among hospitalized patients with haematological malignancies: clinical features, epidemics and outcomes. *J. Hosp. Infect.* 64:169–176.

107. Park, D. J., J. C. Yun, J. E. Baek, E. Y. Jung, D. W. Lee, M.-A. Kim, and S.-H. Chang. 2006. Relapsing *Bacilluslicheniformis* peritonitis in a continuous ambulatory peritoneal dialysis patient. *Nephrology* (Carlton) 11:21–22.
108. Patel, R., K. Piper, F. R. Cockerill, J. M. Steckelberg, and A. A. Yousten. 2000. The biopesticide *Paenibacillus popilliae* has a vancomycin resistance gene cluster homologous to the enterococcal VanA vancomycin gene cluster. *Antimicrob. Agents Chemother.* 44:705–709.
109. Pinna, A., L. A. Sechi, S. Zanetti, D. Esai, G. Delogu, P. Cappuccinelli, and F. Carta. 2001. *Bacillus cereus* keratitis associated with contact lens wear. *Ophthalmology* 108:1830–1834.
110. Priest, F. G., M. Barker, L. W. Baillie, E. C. Holmes, and M. C. Maiden. 2004. Population structure and evolution of the *Bacillus cereus* group. *J. Bacteriol.* 186:7959–7970.
111. Qi, Y., G. Patra, X. Liang, L. E. Williams, S. Rose, R. J. Redkar, and V. G. DelVecchio. 2001. Utilization of the *rpoB* gene as a specific chromosomal marker for real-time PCR detection of *Bacillus anthracis*. *Appl. Environ. Microbiol.* 67:3720–3727.
112. Quinn, C. P., V. A. Semenova, C. M. Elie, S. Romero-Steiner, C. Greene, H. Li, K. Stamey, E. Steward-Clark, D. S. Schmidt, E. Mothershed, J. Pruckler, S. Schwartz, R. F. Benson, L. O. Helsel, P. F. Holder, S. E. Johnson, M. Kellum, T. Messmer, W. L. Thacker, L. Besser, B. D. Plikaytis, T. H. Taylor, Jr., A. E. Freeman, K. J. Wallace, P. Dull, J. Sejvar, E. Bruce, R. Moreno, A. Schuchat, J. R. Lingappa, S. K. Martin, J. Walls, M. Bronsdon, G. M. Carlone, M. Bajani-Ari, D. A. Ashford, D. S. Stephens, and B. A. Perkins. 2002. Specific, sensitive, and quantitative enzyme-linked immunosorbent assay for human immunoglobulin G antibodies to anthrax toxin protective antigen. *Emerg. Infect. Dis.* 8:1103–1110.
113. Quinn, C. P., P. M. Dull, V. Semenova, H. Li, S. Crotty, T. H. Taylor, E. Steward-Clark, K. L. Stamey, D. S. Schmidt, K. W. Stinson, A. E. Freeman, C. M. Elie, S. K. Martin, C. Greene, R. D. Aubert, J. Glidewell, B. A. Perkins, R. Ahmed, and D. S. Stephens. 2004. Immune responses to *Bacillus anthracis* protective antigen in patients with bioterrorism-related cutaneous or inhalation anthrax. *J. Infect. Dis.* 190:1228–1236.
114. Ramos-Esteban, J. C., J. J. Servat, S. Tauber, and F. Bia. 2006. *Bacillus megaterium* delayed onset lamellar keratitis after LASIK. *J. Refract. Surg.* 22:309–312.
115. Rider, T. H., M. S. Petrovick, F. E. Nargi, J. D. Harper, E. D. Schwoebel, R. H. Mathews, D. J. Blanchard, L. T. Bortolin, A. M. Young, J. Chen, and M. A. Hollis. 2003. A B cell-based sensor for rapid identification of pathogens. *Science* 301:213–215.
116. Roux, V., and D. Raoult. 2004. *Paenibacillus massiliensis* sp. nov., *Paenibacillus sanguinis* sp. nov. and *Paenibacillus timonensis* sp. nov., isolated from blood cultures. *Int. J. Syst. Evol. Microbiol.* 54:1049–1054.
117. Roux, V., L. Fenner, and D. Raoult. 2008. *Paenibacillus provencensis* sp. nov., isolated from human cerebrospinal fluid, and *Paenibacillus urinalis* sp. nov., isolated from human urine. *Int. J. Syst. Evol. Microbiol.* 58:682–687.
118. Rubinstein, I., and G. W. Pedersen. 2002. *Bacillus* species are present in chewing tobacco sold in the United States and evoke plasma exudation from the oral mucosa. *Clin. Diagn. Lab. Immunol.* 9:1057–1060.
119. Saile, E., and T. M. Koehler. 2006. *Bacillus anthracis* multiplication, persistence, and genetic exchange in the rhizosphere of grass plants. *Appl. Environ. Microbiol.* 72:3168–3174.
120. Schneemann, A., and M. Manchester. 2009. Anti-toxin antibodies in prophylaxis and treatment of inhalation anthrax. *Future Microbiol.* 4:35–43.
121. Sejvar, J. J., F. C. Tenover, and D. S. Stephens. 2005. Management of anthrax meningitis. *Lancet Infect. Dis.* 5:287–295.
122. Shieh, W. J., J. Guarner, C. Paddock, P. Greer, K. Tatti, M. Fischer, M. Layton, M. Philips, E. Bresnitz, C. P. Quinn, T. Popovic, B. A. Perkins, S. R. Zaki, and the Anthrax Bioterrorism Investigation Team. 2003. The critical role of pathology in the investigation of bioterrorism-related cutaneous anthrax. *Am. J. Pathol.* 163:1901–1910.
123. Shlyakhov, E., and E. Rubinstein. 1994. Human live anthrax vaccine in the former USSR. *Vaccine* 12:727–730.
124. Sirisanthana, T., and A. E. Brown. 2002. Anthrax of the gastrointestinal tract. *Emerg. Infect. Dis.* 8:649–651.
125. Sohni, Y., S. Kanjilal, and V. Kapur. 2008. Performance evaluation of five commercial real-time PCR reagent systems using TaqMan assays for *B. anthracis* detection. *Clin. Biochem.* 41:640–644.
126. Sorokin, A., B. Candelon, K. Guilloux, N. Galleron, N. Wackerow-Kouzova, S. D. Ehrlich, D. Bourguet, and V. Sanchis. 2006. Multiple-locus sequence typing analysis of *Bacillus cereus* and *Bacillus thuringiensis* reveals separate clustering and a distinct population structure of psychrotrophic strains. *Appl. Environ. Microbiol.* 72:1569–1578.
127. Spinosa, M. R., F. Wallet, R. J. Courcol, and M. R. Oggioni. 2000. The trouble in tracing opportunistic pathogens: cholangitis due to *Bacillus* in a French hospital caused by a strain related to an Italian product? *Microb. Ecol. Health. Dis.* 12:99–101.
128. Stenfor Arnesen, L. P., A. Fagerlund, and P. E. Granum. 2008. From soil to gut: *Bacillus cereus* and its food poisoning toxins. *FEMS Microbiol. Rev.* 32:579–606.
129. Stern, E. J., K. B. Uhde, S. V. Shadomy, and N. Messonnier. 2008. Conference report on public health and clinical guidelines for anthrax. *Emerg. Infect. Dis.* 14:e1.
130. Stickel, F., S. Droz, E. Patsenker, K. Bögli-Stuber, B. Aebi, and S. Leib. 2009. Severe hepatotoxicity following ingestion of Herbalife nutritional supplements contaminated with *Bacillus subtilis*. *J. Hepatol.* 50:111–117.
131. Stratilo, C. W., C. T. Lewis, L. Bryden, M. R. Mulvey, and D. Bader. 2006. Single-nucleotide repeat analysis for subtyping *Bacillus anthracis* isolates. *J. Clin. Microbiol.* 44:777–782.
132. Sue, D., C. K. Marston, A. R. Hoffmaster, and P. P. Wilkins. 2007. Genetic diversity in a *Bacillus anthracis* historical collection (1954 to 1988). *J. Clin. Microbiol.* 45:1777–1782.
133. Tang, S., M. Moayeri, Z. Chen, H. Harma, J. Zhao, H. Hu, R. H. Purcell, S. H. Leppa, and I. K. Hewlett. 2009. Detection of anthrax toxin by an ultrasensitive immunoassay using europium nanoparticles. *Clin. Vaccine Immunol.* 16:408–413.
134. Tena, D., J. A. Martinez-Torres, M. T. Perez-Pomata, J. A. Sáez-Nieto, V. Rubio, and J. Bisquert. 2007. Cutaneous infection due to *Bacillus pumilus*: report of 3 cases. *Clin. Infect. Dis.* 44:40–42.
135. Thorsen, L., B. M. Hansen, K. F. Nielsen, N. B. Hendriksen, R. K. Phipps, and B. B. Budde. 2006. Characterization of emetic *Bacillus weihenstephanensis*, a new cereulide-producing bacterium. *Appl. Environ. Microbiol.* 72:5118–5121.
136. Tourasse, N. J., E. Helgason, O. A. Okstad, I. K. Hegna, and A. B. Kolsto. 2006. The *Bacillus cereus* group: novel aspects of population structure and genome dynamics. *J. Appl. Microbiol.* 101:579–593.
137. Tourasse, N. J., and A.-B. Kolstø. 2008. SuperCAT: a supertree database for combined and integrative multilocus sequence typing analysis of the *Bacillus cereus* group of bacteria (including *B. cereus*, *B. anthracis* and *B. thuringiensis*). *Nucleic Acids Res.* 36:D461–D468.
138. Tournier, J. N., R. G. Ulrich, A. Quesnel-Hellmann, M. Mohamadzadeh, and B. G. Stiles. 2009. Anthrax, toxins and vaccines: a 125-year journey targeting *Bacillus anthracis*. *Expert Rev. Anti-Infect. Ther.* 7:219–236.
139. Tuladhar, R., S. K. Patole, T. H. Koh, R. Norton, and J. S. Whitehall. 2000. Refractory *Bacillus cereus* infection in a neonate. *Int. J. Clin. Pract.* 54:345–347.
140. Turnbull, P. C. B. (ed.). 2008. *Anthrax in Humans and Animals*, 4th ed. World Health Organization, Geneva, Switzerland. <http://www.who.int/csr/resources/publications/AnthraxGuidelines2008>

141. Turnbull, P. C. B., N. M. Sirianni, C. I. LeBron, M. N. Samaan, F. N. Sutton, A. E. Reyes, and L. F. Peruski. 2004. MICs of selected antibiotics for *Bacillus anthracis*, *Bacillus cereus*, *Bacillus thuringiensis*, and *Bacillus mycoides* from a range of clinical and environmental sources as determined by the Etest. *J. Clin. Microbiol.* **42**:3626–3634.
142. Turnbull, P. C. B., D. A. Frawley, and R. L. Bull. 2007. Heat activation/shock temperatures for *Bacillus anthracis* spores and the issue of spore plate counts versus true numbers of spores. *J. Microbiol. Methods* **68**:353–357.
143. Van Ert, M. N., S. A. Hofstadler, Y. Jiang, J. D. Busch, D. M. Wagner, J. J. Drader, D. J. Ecker, J. C. Hannis, L. Y. Huynh, J. M. Schupp, T. S. Simonson, and P. Keim. 2004. Mass spectrometry provides accurate characterization of two genetic marker types in *Bacillus anthracis*. *BioTechniques* **37**:642–644, 646, 648 passim.
144. Van Ert, M. N., W. R. Easterday, L. Y. Huynh, R. T. Okinaka, M. E. Hugh-Jones, J. Ravel, S. R. Zanecki, T. Pearson, T. S. Simonson, J. M. U'Ren, S. M. Kachur, R. R. Leadem-Dougherty, S. D. Rhoton, G. Zinser, J. Farlow, P. R. Coker, K. L. Smith, B. Wang, L. J. Kenefic, C. M. Fraser-Liggett, D. M. Wagner, and P. Keim. 2007. Global genetic population structure of *Bacillus anthracis*. *PLoS ONE* **2**:e461.
145. Van Ert, M. N., W. R. Easterday, T. S. Simonson, J. M. U'Ren, T. Pearson, L. J. Kenefic, J. D. Busch, L. Y. Huynh, M. Dukerich, C. B. Trim, J. Beaudry, A. Welty-Bernard, T. Read, C. M. Fraser, J. Ravel, and P. Keim. 2007. Strain-specific single-nucleotide polymorphism assays for the *Bacillus anthracis* Ames strain. *J. Clin. Microbiol.* **45**:47–53.
146. Vijaikumar, M., D. M. Thappa, and K. Karthikeyan. 2002. Cutaneous anthrax: an endemic outbreak in South India. *J. Trop. Pediatr.* **48**:225–226.
147. Walsh, J., J. N. Pesik, C. P. Quinn, V. Urdaneta, C. A. Dykewicz, A. E. Boyer, J. Guarner, P. Wilkins, K. J. Norville, J. R. Barr, S. R. Zaki, J. B. Patel, S. P. Reagan, J. L. Pirkle, T. A. Treadwell, N. Rosenstein Messonnier, L. D. Rotz, R. F. Meyer, and D. S. Stephens. 2007. A case of naturally acquired inhalation anthrax: clinical care and analyses of anti-protective antigen immunoglobulin G and lethal factor. *Clin. Infect. Dis.* **44**:968–971.
148. Weber, D. J., S. M. Saviteer, W. A. Rutala, and C. A. Thomann. 1988. In vitro susceptibility of *Bacillus* spp. to selected antimicrobial agents. *Antimicrob. Agents Chemother.* **32**:642–645.
149. Whiteaker, J., J. Karns, C. Fenselau, and M. L. Perdue. 2004. Analysis of *Bacillus anthracis* spores in milk using mass spectrometry. *Foodborne Pathog. Dis.* **1**:185–194.
150. Whiting, G. C., S. Rijpkema, T. Adams, and M. J. Corbel. 2004. Characterization of adsorbed anthrax vaccine by two-dimensional gel electrophoresis. *Vaccine* **22**:4245–4251.
151. Yakupogullari, Y., and M. Koroglu. 2007. Nosocomial spread of *Bacillus anthracis*. *J. Hosp. Infect.* **66**:401–402.
152. Zahavy, E., M. Fisher, A. Bromberg, and U. Olshevsky. 2003. Detection of frequency resonance energy transfer pair on double-labeled microsphere and *Bacillus anthracis* spores by flow cytometry. *Appl. Environ. Microbiol.* **69**:2330–2339.
153. Zwick, M. E., F. McAfee, D. J. Cutler, T. D. Read, J. Ravel, G. R. Bowman, D. R. Galloway, and A. Mateczun. 2005. Microarray-based resequencing of multiple *Bacillus anthracis* isolates. *Genome Biol.* **6**:R10.